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Chemotactic and mitogenic stimuli of neuronal apoptosis in patients with medically intractable temporal lobe epilepsy

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Abstract

To identify the upstream signals of neuronal apoptosis in patients with medically intractable temporal lobe epilepsy (TLE), we evaluated by immunohistochemistry and confocal microscopy brain tissues of 13 TLE patients and 5 control patients regarding expression of chemokines and cell-cycle proteins. The chemokine RANTES (CCR5) and other CC-chemokines and apoptotic markers (caspase-3, -8, -9) were expressed in lateral temporal cortical and hippocampal neurons of TLE patients, but not in neurons of control cases. The chemokine RANTES is usually found in cytoplasmic and extracellular locations. However, in TLE neurons, RANTES was displayed in an unusual location, the neuronal nuclei. In addition, the cell-cycle regulatory transcription factor E2F1 was found in an abnormal location in neuronal cytoplasm. The pro-inflammatory enzyme cyclooxygenase-2 and cytokine interleukin-1β were expressed both in neurons of patients suffering from temporal lobe epilepsy and from cerebral trauma. The vessels showed fibrin leakage, perivascular macrophages and expression of IL-6 on endothelial cells. In conclusion, the cytoplasmic effects of E2F1 and nuclear effects of RANTES might have novel roles in neuronal apoptosis of TLE neurons and indicate a need to develop new medical and/or surgical neuroprotective strategies against apoptotic signaling by these molecules. Both RANTES and E2F1 signaling are upstream from caspase activation, thus the antagonists of RANTES and/or E2F1 blockade might be neuroprotective for patients with medically intractable temporal lobe epilepsy. The results have implications for the development of new medical and surgical therapies based on inhibition of chemotactic and mitogenic stimuli of neuronal apoptosis in patients with medically intractable temporal lobe epilepsy.
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1. Introduction

The complex signaling pathways leading to neuronal apoptosis in epilepsy have not been completely elucidated. Seizures stimulate calcium influx, glutamate receptor activation and/or release of death receptor ligands [1] and may provoke neuronal death by mitochondrial [2] and death receptor pathways [3,4]. The E2F family (E2F1–E2F6) is responsible for regulating cell cycle progression; however, E2F1 is also able to induce cell death through several mechanisms [5]. The transcription factor, E2F1, and the interacting pocket-binding protein, Rb, are implicated in the death of neurons in neurodegenerative disorders [6,7]. E2F1 protein expression is increased in the neuronal cytoplasm of involved brain regions in HIV encephalitis, simian-immunodeficiency virus (SIV) encephalitis [8], Alzheimer disease [9], amyotrophic lateral sclerosis [10], and Parkinson's disease [11].

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Affected neurons in Parkinson's disease display altered distribution of phosphorylated retinoblastoma protein (Rb) [12]. In amyotrophic lateral sclerosis, E2F1 is redistributed into the cytoplasm of motor neurons and the transcriptional regulator Rb is hyperphosphorylated [10]. The International League Against Epilepsy defines medically intractable epilepsy as the failure of two tolerated, appropriately chosen and used antiepileptic medications schedules (either monotherapy or combination therapy) to achieve sustained seizure freedom [12a]. The expression and distribution of E2F1 and Rb have not been previously investigated in brain tissue of patients with medically intractable temporal lobe epilepsy.

Inflammation leads to apoptosis through induction of inflammatory cytokines and chemokines. Inflammatory cytokines including interleukin-1β (IL-1β) are not constitutively expressed in normal brain [13] but are detected in a wide range of neurodegenerative disorders [14]. Cytokine expression has been noted in autopsy tissues of patients with temporal lobe epilepsy [15] and rodent brain tissues after seizure induction [16]. The cytokine IL-1β may induce pro-apoptotic and excitatory signals, which lead to long-lasting changes in gene transcription [17]. In animal models of epileptogenesis, activation of the IL-1β system is associated with neurodegeneration and blood–brain barrier breakdown [18]. Anti-inflammatory drugs are considered for use in epilepsy both for their anticonvulsant activity and modulation of gene transcription [19]. Vagus nerve stimulation has been shown to have immune rebalancing functions which may be related to its antiseizure action [20]. However, expression of IL-1, TNF-alpha and IL-10 in the brain may be associated with cell injury other than that associated with seizures and these cytokines may in certain situations have neuroprotective effects [21,22].

Chemokines are chemotactic proteins classified into four subfamilies known as CXC-, CC-, C-, and CXXXC-chemokines [23]. In the central nervous system, chemokines have additional functions, including control of neural plasticity by CCL5 [24], a role of CCL5–CCR5 in inflammation and apoptosis, and a dichotomous role of CXCR4, which has positive neurodevelopmental effects through its ligand SD F-1 [25] and neurotoxic effects mediated by the HIV-1 protein gp120 [26]. Chemokines may lead to neuronal death through E2F1 signaling [27].

In this study, we examine upstream signals for apoptosis, including inflammatory signals by chemokines, cytokines and cyclooxygenase-2 (COX-2) and the cell cycle transcription factor, E2F1. The results suggest that apoptosis in neurons of patients with medically intractable temporal lobe epilepsy ("TLE neurons") may be induced by a combination of chemotactic and mitogenic stimuli. These findings have implications for tailoring the development of new medical and surgical therapies targeted towards specific chemotactic and mitogenic stimuli of neuronal apoptosis in patients with medically intractable temporal lobe epilepsy.

2. Materials and methods

2.1. Surgical tissues

The Institutional Review Boards at the University of Arizona and UCLA approved the protocol of the study and the Human Consent. The study involved 13 TLE patients, 18–50 years old with the average preoperative seizure frequency ranging from 0.02 to 18 seizures per day (rare seizures, seizure frequency $\leq$0.1 seizures per day, in 6 patients; frequent seizures, seizure frequency >0.1 seizure per day, in 7 patients), temporal lobe origin of seizures and clinical phenomenology of complex partial seizures. All patients in this study underwent en bloc anterior temporal lobectomy and amygdalohippocampectomy after preoperative planning and seizure focus localization to a single temporal lobe in accordance with previously described technique [28]. Briefly, the anterior temporal lobectomy technique involved en bloc resection of the lateral temporal cortex followed by dissection of superior temporal gyrus white matter using subpial technique, exposure of the temporal horn, and identification of the amygdala and hippocampus using the choroidal fissure as the superior-most landmark for en bloc resection of the hippocampus. The neurosurgical specimens were removed within 30–45 min from the beginning of the operation. Postoperatively, eight patients became seizure-free, 5 significantly improved and one improved in accordance with previously described seizure outcome criteria [28] (Table 1). Lateral temporal cortical tissues included surgical specimens of one trauma and one brain tumor (glioblastoma multiforme) case, and three postmortem cases of non-epileptic temporal lobe tissues. Samples were split with sterile scalpels in an aseptic environment for processing into 10% buffered formalin, which was kept at room temperature, and O.C.T. compound, which was placed into liquid nitrogen for immediate freezing. Formalin samples were transferred into 70% ETOH the next morning and sent for routine paraffin embedding. Samples were cut at 5 μm slices for immunohistochemistry. Frozen blocks were transferred to −80 °C until sectioned at 5 μm for immunofluorescence.

2.2. Immunohistochemistry (IHC)

IHC was performed using the single or double Envision technique (with rabbit and mouse antibodies) or the LSAB 2 technique (with goat antibody) (both from DAKO, Carpinteria, CA) as previously described [29]. Briefly, the tissue sections were deparaffinized, subjected to antigen retrieval by steam in DAKO antigen retrieval solution, blocked with dual endogenous block solution followed by serum-free protein block solution (both DAKO), stained in the Sequenza apparatus with the primary antibody in the single Envision technique (DAKO) and stained additionally with the second primary antibody in the double Envision technique. The primary antibodies (at 5 or 10 μg/ml) were rabbit anti-RANTES (Torrey Pines Biolabs Inc.), mouse-anti-RANTES (BioSource),