Tissue Expressions of Soluble Human Epoxide Hydrolase-2 Enzyme in Patients with Temporal Lobe Epilepsy

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■ OBJECTIVE: **We sought to simply demonstrate how levels of soluble human epoxide hydrolase-2 show changes in both temporal the cortex and hippocampal complex in patients with temporal lobe epilepsy.**

■ METHODS: **A total of 20 patients underwent anterior temporal lobe resection due to temporal lobe epilepsy. The control group comprised 15 people who died in traffic accidents or by falling from a height, and their autopsy findings were included. Adequately sized temporal cortex and hippocampal samples were removed from each patient during surgery, and the same anatomic structures were removed from the control subjects during the autopsy procedures. Each sample was stored at −80°C as rapidly as possible until the enzyme assay.**

■ RESULTS: **The temporal cortex in the epilepsy patients had a significantly higher enzyme level than did the temporal cortex of the control group ($P = 0.03$). Correlation analysis showed that as the enzyme level increases in the temporal cortex, it also increases in the hippocampal complex ($r^2 = 0.06$, $P = 0.00001$). More important, enzyme tissue levels showed positive correlations with seizure frequency in both the temporal cortex and hippocampal complex in patients ($r^2 = 0.7$, $P = 0.00001$ and $r^2 = 0.4$, $P = 0.003$, respectively). The duration of epilepsy was also positively correlated with the hippocampal enzyme level ($r^2 = 0.06$, $P = 0.00001$).**

■ CONCLUSIONS: **Soluble human epoxy hydrolase enzyme-2 is increased in both lateral and medial temporal tissues in temporal lobe epilepsy. Further studies should be conducted as inhibition of this enzyme has resulted in a significant decrease in or stopping of seizures and attenuated neuroinflammation in experimental epilepsy models in the current literature.**

INTRODUCTION

Epilepsy is one of the most common chronic and neurodegenerative neurologic disorders characterized by recurrent abnormal electrical discharges from the affected brain. Among epilepsy disorders, temporal lobe epilepsy (TLE) is the single most common focal epilepsy and is characterized by structural and functional changes in the temporal lobe, especially the hippocampus, called hippocampal sclerosis.

Recent studies have shown that neuroinflammation could play a critical role in either structural or functional changes in the hippocampus. During neuroinflammation, a cascade of pathologic processes is initiated by the activation of microglia and astrocytes, producing proinflammatory molecules, namely interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor-alpha.[1,2] These molecules activate a cascade in which phospholipases act at the center to produce arachidonic acids (AAs). In the brain parenchyma, released AAs are converted into a lipid metabolite,

Key words
- Epoxide hydrolase
- Inflammation
- Temporal lobe epilepsy

Abbreviations and Acronyms
AA: Arachidonic acid
AED: Antiepileptic drug
EEG: Electroencephalography
EET: Epoxyeicosatrienoic acid
GABA: Gamma-aminobutyric acid
IL-1β: Interleukin-1 beta
IL-6: Interleukin-6
sHEH-2: Soluble human epoxide hydrolase-2
TLE: Temporal lobe epilepsy

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epoxyeicosatrienoic acids (EETs), by the action of cytochrome P450 epoxygenases.[3] It is clear from the current literature that EETs have important actions such as regulation of cerebral blood flow[4] and are antiinflammatory and antiapoptotic molecules.[5] In ischemic animal models, increased action of cytochrome P450 epoxygenases and EETs protected cerebral tissue against ischemic injury and decreased neuroinflammation, suggesting their antineuroinflammatory roles.[4,6,7]

Metabolic conversion of EETs into their less active form, dihydroxyeicosatrienoic acids, is possible by the action of a key enzyme, soluble human epoxide hydrolase-2 (sHEH-2).[8] It has been suggested that inhibition of sHEH-2 increases levels of EETs and ameliorates neuronal injury caused by cerebral ischemia.[3,6,9] More importantly, inhibition of sHEH-2 selectively modulates gamma-aminobutyric acid—mediated (GABA) neurotransmission to delay the onset of seizure by decreasing neuronal excitability.[10] It has been demonstrated in mouse models of TLE that sHEH-2 activity regulates neuroinflammation and seizure generation.[11]

Unfortunately, little information is known about sHEH-2 in TLE, and there has been only 1 report related to TLE in which effects of pharmacologic and genetic inhibition of sHEH-2 in a mouse model of TLE have been studied.[11] The present study is the first to show tissue levels of sHEH-2 in human patients with TLE. The aim is to show how levels of this enzyme exhibit changes in both the temporal cortex and hippocampal complex compared with the same anatomic structures obtained during autopsy procedures of the victims who died as a result of different causes.

MATERIALS AND METHODS

This study was performed with the collaboration of the Departments of Neurosurgery and Biochemistry. Patients who had TLE histopathologically proven and who signed the informed consent were included. All patients or next of kin were fully informed, and ethical approval for this study was obtained from the local ethics committee.

Patients

A total of 20 patients who underwent anterior temporal resection due to TLE were included. All patients were discussed in our local epilepsy meetings, and routine presurgical measurements were taken. All patients had scalp electroencephalography (EEG) including ictal/interictal findings, head magnetic resonance imaging, and neuropsychologic tests. In cases of discordant data among the clinical semiology, EEG, and/or magnetic resonance imaging findings, invasive EEG monitoring (depth electrode) and/or positron emission tomography was performed. After completion of presurgical measurements, the risk/benefit ratio of surgery was explained to all patients and if they approved, epilepsy surgery was performed. All patients were followed up after surgery at regular intervals by our expert epilepsy team with respect to surgical and neurologic outcomes.

Controls

The control group comprised 15 people who died in traffic accidents or by falling from a height; all underwent autopsy procedures in the Department of Forensic Medicine. No subject showed gross pathology in the brain during the autopsy procedures.

Specimen Handling

Adequately sized temporal cortex and hippocampal samples were removed from each patient during surgery. Temporal cortex and hippocampal tissues from the control subjects were removed during the autopsies, which were performed within 4 hours of death. Each sample was stored at −80°C as rapidly as possible until required for sHEH-2 assay.

Assay of sHEH-2 Levels

Measurements of sHEH-2 were performed by a sandwich enzyme-linked immunosorbent assay (Cusabio, Hubei, China). Protein measurements were performed by immunoturbidimetric assay (Beckman Coulter, Pasadena, California, USA), and results were expressed as μg/g protein tissue. Tissue samples that were stored at −80°C were brought to room temperature, and after cleaning, the tissues were weighed (8.41 ± 19.62). All the tissue samples were washed with a 0.1 M phosphate buffer (pH 7.4), plus 1 mL, and subsequently homogenized using a Wiggenhauser D130 handheld homogenizer (Selangor, Malaysia). The samples were centrifuged for 5 minutes at 5000 g and 4°C. The supernatants were analyzed to determine the levels of sHEH-2.

STATISTICAL ANALYSIS

We used a commercially available statistical software package (SPSS version 14.0 Inc., Chicago, Illinois, USA) for all statistical analyses. The mean ± SD was calculated for each parameter. The nonparametric Mann-Whitney U test and chi-square test were used for appropriate comparisons. Differences were considered statistically significant if $P < 0.05$.

RESULTS

The patient group consisted of 9 women and 11 men with a mean age of 31.2 ± 10.8 years. Mean seizure onset and duration of seizure were noted as 14.0 ± 10.8 and 17.9 ± 11.7 years, respectively. The control group had 10 women and 5 men with a mean age of 38.0 ± 10.7 years. Comparisons between patients and control subjects with respect to gender (χ^2 test; $P = 0.65$) and mean age (Mann-Whitney U test; $P = 0.07$) showed no difference. No patient had a family history of head trauma as a risk factor for epilepsy, and half of the patients specified a risk factor such as febrile convulsions in their medical history. Mean seizure frequency/month before surgery was found to be 5.55 ± 6.5; ranging from 1—30 and all were on antiepileptic drugs (AEDs) before surgery.

Following completion of preoperative work-up, anterior temporal resections together with mesial temporal structures (amygdala and hippocampal/parahippocampal complex) were performed, and the extent of resection depended primarily on the results of neuropsychologic tests. Because the aim of this study is not to present detailed surgical and clinical outcomes of the patients, tissue enzyme levels will be the focus of the results section.

Regarding sHEH-2 levels, hippocampal complex in both patients and control subjects showed higher levels compared with