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Seizure-Related Neuronal Injury

A study of neuron-specific enolase,
S-100b protein and tau protein

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CONTENTS

ABSTRACT

Both experimental and clinical studies have established that status epilepticus (SE) induces neuronal injury. Whether brief recurrent seizures damage the brain is controversial. The markers of brain damage, neuron-specific enolase (NSE) and S-100b protein (S-100b) have been used to assess the primary injury as well as to predict outcome after various brain-damaging conditions. The levels of NSE may increase after SE, but the question whether a single tonic-clonic or complex partial seizure induces elevation of NSE and S-100b is still open. Cerebrospinal fluid (CSF) tau protein measurements have been suggested for the diagnosis of Alzheimer's disease, and total tau (T-tau) may also be a marker of axonal damage and neuronal degeneration. CSF tau levels have not been previously measured after epileptic seizures. Electroconvulsive therapy (ECT) is the most effective treatment for patients with severe depression. An adequate generalized epileptic seizure is necessary for a therapeutic response; therefore, possibility of neuronal loss or dysfunction in some patients still raises concerns. At present, studies using biomarkers in patients treated with ECT are scant.

The levels of NSE, S-100b and tau were measured in different patient groups with seizures. Patients with newly onset seizures, patients with refractory epilepsy, and patients with generalized tonic-clonic seizures with various etiologies were included in the study. Serum and CSF samples were collected shortly after seizures. Serial serum samples of NSE and S-100b were obtained also from patients treated with ECT.

CSF NSE or S-100b concentrations were not increased in patients with single uncomplicated newly onset seizures. On the other hand, serial serum measurements of both NSE and S-100b levels showed elevations in patients with refractory temporal lobe epilepsy (TLE), whereas in extratemporal epilepsies (XTLE) the changes were not significant. CSF tau levels after seizures were not increased in patients with idiopathic or probably symptomatic (cryptogenic) epilepsy, and abnormal CSF tau levels were only found in patients with either acute or remote symptomatic seizures. Serial serum measurements of NSE or S-100b did not show significant changes in patients treated with ECT. However, a transient elevation of S-100b after ECT in four out of ten patients was observed, and high S-100b levels at 2 h and 6 h after ECT correlated with the treatment response.

Our results imply that single uncomplicated seizures do not increase the levels of these biomarkers indicating absence of seizure-induced neuronal injury. In TLE, increased levels of NSE and S-100b suggest that in refractory TLE with repeated seizures the sensitivity to damage is different, and the damage induced by recurrent brief seizures over the years contributes to the progression of the disorder. S-100b has a dual role as a marker of either glial damage or glial activation. Our results of S-100b levels after ECT do not indicate neuronal injury but rather demonstrate glial activation induced by ECT. The activation could be neurotrophic in nature and the antidepressant effect of ECT may be mediated by this glial activation.

TIIVISTELMÄ

Sekä kokeelliset että kliiniset tutkimukset ovat osoittaneet, että status epilepticus (SE) aiheuttaa hermosolujen vaurioitumista. On epäselvää, aiheuttavatko myös lyhytkestoiset toistuvat kohtaukset vauriota. Aivovaurion merkkiaineita, neuronispesifistä enolaasia (NSE) ja S-100b-proteiinia (S-100b), on käytetty aivoja vaurioittavissa tiloissa vaurion asteen arvioinnissa ja toipumisen ennustamisessa. NSE:n pitoisuudet voivat nousta SE:n jälkeen, mutta ei tiedetä, aiheuttavatko yksittäiset toonis-klooniset tai monimuotoiset paikallisalkuiset kohtaukset NSE:n tai S-100b:n nousua. Aivo-selkäydinnesteestä (cerebrospinal fluid, CSF) mitattava tau-proteiini on käytössä Alzheimerin taudin diagnostiikassa, mutta kokonais-tau (total tau, T-tau) voi olla myös aksonaalisen vaurion ja neurodegeneraation merkkiaine. CSF tau-pitoisuuksia ei ole aiemmin mitattu epileptisten kohtausten jälkeen. Sähköhoito (electroconvulsive therapy, ECT) on tehokkain hoitomuoto vakavassa masennuksessa. Hoidollisen tehon saavuttamiseksi vaaditaan riittävä yleistynyt epileptinen kohtaus, jonka vuoksi hermosolujen vaurioitumisen tai toimintahäiriön mahdollisuus on edelleen huolenaihe. Biomarkkereilla tehtyjä tutkimuksia ECT-hoitoa saavilla potilailla on toistaiseksi vähän.

Epileptisen kohtauksen saaneilta potilailta mitattiin seerumin tai CSF:n NSE:n, S-100b:n ja taun pitoisuudet pian kohtauksen jälkeen. Potilasryhminä olivat vastasairastuneet potilaat, vaikeahoitoista epilepsiaa sairastavat potilaat ja potilaat, joilla yleistynyt toonis-klooninen kohtaus aiheutui erilaisista syistä. Lisäksi keräsimme sarjan seerumin NSE- ja S-100b-näytteitä ECT-hoitoa saavilta potilailta.

CSF:n NSE- tai S-100b-pitoisuudet eivät nousseet vastasairastuneilla potilailla, joilla oli yksittäinen, komplisoitumaton kohtaus. Toisaalta seerumin NSE- ja S-100b-pitoisuudet nousivat vaikeahoitoista temporaaliepilepsiaa (TLE), mutta ei extratemporaaliepilepsiaa sairastavilla. CSF:n tau-pitoisuudet eivät nousseet idiopaattista tai kryptogeenista epilepsiaa sairastavilla. Poikkeavia tau-arvoja todettiin vain potilailla, joilla oli joko akuutti tai kaukaisempi symptomaattinen syy epileptisiin kohtauksiin. Seerumin NSE- tai S-100b-sarjamittaukset eivät merkittävästi muuttuneet ECT-potilailla. Lyhytkestoisia S-100b:n nousuja kuitenkin todettiin neljällä potilaalla kymmenestä ja korkea S-100b-pitoisuus 2 ja 6 tunnin kohdalla korreloi hoitotuloksiin.

Tuloksemme viittaavat siihen, että yksittäinen, komplisoitumaton kohtaus ei aiheuta biomarkkereiden pitoisuuksien nousua, eikä löydös viittaa kohtauksen aiheuttamaan hermosolun vaurioon. TLE-potilailla NSE- ja S-100b-pitoisuuksien nousu viittaa vaikeahoitoisen TLE:n erilaiseen vaurioitumisherkkyyteen, ja vaurio, jonka toistuvat lyhyet kohtaukset aiheuttavat, voi vaikuttaa vuosien saatossa taudin etenemiseen. S-100b-tason nousu heijastaa joko glian vauriota tai aktivaatiota. S-100b-määritykset ECT-potilailla eivät viittaa hermoston vaurioon vaan sopivat ECT:n indusoimaan glia-aktivaatioon. Aktivaatio voi olla luonteeltaan neurotrooppinen ja ECT:n hoitovaste saattaa välittyä tämän glian aktivoitumisen kautta.

ABBREVIATIONS

AD	Alzheimer's disease
AED	antiepileptic drug
BBB	blood-brain barrier
BDI	Beck Depression Inventory
BDNF	brain-derived neurotrophic factor
CJD	Creutzfeldt - Jakob disease
CNS	central nervous system
CPS	complex partial seizure
CPSE	complex partial status epilepticus
CSF	cerebrospinal fluid
CT	computed tomography
ECS	electroconvulsive stimulation
ECT	electroconvulsive therapy
EEG	electroencephalography
EMG	electromyogram
GABA	gamma aminobutyric acid
GFAP	glial fibrillary acidic protein
GTCS	generalized tonic-clonic seizure
GTCSE	generalized tonic-clonic status epilepticus
HS	hippocampal sclerosis
ILAE	International League Against Epilepsy
LP	lumbar puncture
MADRS	Montgomery-Åsberg Depression Rating Scale

MBP	myelin basic protein
MDD	major depressive disorder
MMSE	Mini Mental State Examination
MRI	magnetic resonance imaging
NSE	neuron-specific enolase
P-tau	phosphorylated tau protein
PTZ	pentylenetetrazol
S-100b	S-100b protein
SGTCS	secondary generalized tonic-clonic seizure
SE	status epilepticus
SPS	simple partial seizure
SUDEP	sudden unexpected death in epilepsy
T-tau	total tau protein
TBI	traumatic brain injury
TLE	temporal lobe epilepsy
XTLE	extratemporal epilepsy

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original communications referred to in the text by Roman numerals **I-IV**:

I Palmio J, Peltola J, Vuorinen P, Laine S, Suhonen J, Keränen T (2001). Normal CSF neuron-specific enolase and S-100 protein levels in patients with recent non-complicated tonic-clonic seizure. J Neurol Sci 183:27-31.

II Palmio J, Keränen T, Alapirtti T, Hulkkonen J, Mäkinen R, Holm P, Suhonen J, Peltola J (2008). Elevated serum neuron-specific enolase in patients with temporal lobe epilepsy: a video-EEG study. Epilepsy Res 81:155-160.

III Palmio J, Suhonen J, Keränen T, Hulkkonen J, Peltola J, Pirttilä T (2009). Cerebrospinal fluid tau as a marker of neuronal damage after epileptic seizure. Seizure, in press

IV Palmio J, Huuhka M, Laine S, Huhtala H, Peltola J, Leinonen E, Suhonen J, Keränen T (2009). Electroconvulsive therapy and biomarkers of neuronal injury and plasticity: Serum levels of neuron-specific enolase and S-100b protein. Psychiatry Res, in press

INTRODUCTION

Epilepsy is a disorder of the brain that affects people of all ages and in every country of the world. It is the most common acquired chronic neurological disorder that affects 1% to 3% of the population, i.e., around 50 million people world wide have epilepsy (Hauser et al. 1996). It is characterized by recurrent seizures, which are physical reactions to sudden, usually brief, electrical discharges in parts of the brain. Characteristics of seizures vary and depend on where in the brain the disturbance starts and how it spreads. Clinical symptoms are transient, such as loss of awareness or consciousness, and disturbances of movement, sensation, mood or mental function. Some epilepsies are idiopathic, probably with genetic background and some are a consequence of a known or suspected symptomatic cause, e.g. brain tumor, stroke or congenital disorder. Acute symptomatic seizures are usually due to acute systemic or cerebral causes or related to alcohol or other substance withdrawal syndrome.

Epilepsy is one of the world's oldest recognized conditions. Fear, misunderstanding, discrimination and social stigma have surrounded epilepsy for centuries. Accounts of and references to epileptic seizures can be found in several ancient scripts, but not until Hippocrates was the origin of epilepsy placed in the brain (Daras et al. 2008). With modern medication, 60-70% of patients with newly diagnosed epilepsy enter long-term remission (Kwan and Brodie 2000). Nevertheless, over 30% of patients continue to have seizures (Kwan and Brodie 2000) and a subset of these patients develop progressive epilepsy with an increase of seizure frequency and cognitive decline (Collaborative Group for the Study of Epilepsy 1992). Thus, some of the stigma continues today in many countries and can impact the quality of life for people with the disorder and their

families. Further, epilepsy clearly increases a person's risk of premature death compared to the general population and sudden unexpected death in epilepsy is the most common seizure related category (Tomson et al. 2008).

For many decades, there has been debate whether epileptic seizures, especially brief recurrent seizures, damage the brain. Both status epilepticus (SE) and recurrent brief seizures can have severe and lasting effects on the brain structure. These effects can either be harmful or protective attempts to counterbalance the pro-epileptic effects (Parent et al. 2008). Various biomarkers have been studied in the context of epilepsy and brain damage. However, no validated marker has yet been established. Neuron-specific enolase (NSE), S-100b protein (S-100b), and more recently tau protein have been used as markers of brain damage in various neurological disorders and conditions. There are reports of NSE and S-100b in the context of epilepsy and brain damage, but their role is controversial (DeGiorgio et al. 1995, Leutmezer et al. 2002). Tau protein has not been previously studied with recent epileptic seizures. An ideal biomarker could help determine the critical duration of epileptic seizures prior to neuronal injury, define subtypes of epilepsy requiring more aggressive interventions and possibly serve as a prognostic tool.

REVIEW OF THE LITERATURE

1. Epileptic seizures and epileptic syndromes

Epileptic seizures are associated with electric discharges in a population of hyperexcitable neurons. Epileptic seizures are usually caused by discharges generated in cortical and hippocampal structures, although subcortical structures are involved in some seizure types. The clinical features of a seizure depend on its site of origin, duration, and propagation (Avanzini and Franceschetti 2003). Seizures are either acute symptomatic or unprovoked. Acute symptomatic seizures occur at the time of a systemic insult or in close temporal association with a brain insult. Unprovoked seizures occur in the absence of triggering factors (Hauser and Beghi 2008). Epilepsies are a heterogeneous group of conditions characterized by the recurrence of spontaneous unprovoked seizures. Together they represent the most common acquired chronic neurological disorder, affecting 1% to 3% of the population (Hauser et al. 1996). The prevalence of epilepsy is 5-9/1000; the cumulative lifetime incidence is approximately 3% (Shneker and Fountain 2003). The latest annual incidence in the total population in Finland is 52.9/100 000 (Sillanpää et al. 2006).

Epileptic seizures are divided into focal and generalized seizures. In focal (partial) seizures the initial activation is limited to part of one cerebral hemisphere, whereas in generalized seizures it involves both hemispheres. Self-limited epileptic seizures are distinguished from SE (Table 1). An epilepsy syndrome is a complex of signs and symptoms that define a unique epilepsy condition (Table 2).

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TABLE 1 The International League Against Epilepsy (ILAE) classification of seizure types (2001)

Self-limited seizure types

Generalized seizures
Tonic-clonic seizures
Clonic seizures
Without tonic features
With tonic features
Typical absence seizures
Atypical absence seizures
Myoclonic absence seizures
Tonic seizures
Spasms
Myoclonic seizures
Eyelid myoclonia
Without absences
With absences
Myoclonic atonic seizures
Negative myoclonus
Atonic seizures
Reflex seizures in generalized epilepsy syndromes

Focal seizures
Focal sensory seizures
With elementary sensory symptoms
With experiential sensory symptoms
Focal motor seizures
With elementary clonic motor signs
With asymmetrical tonic motor seizures
With typical (temporal lobe) automatisms
With hyperkinetic automatisms
With focal negative myoclonus
With inhibitory motor seizures
Gelastic seizures
Hemiclonic seizures
Secondarily generalized seizures
Reflex seizures in focal epilepsy syndromes

Continuous seizure types

Generalized status epilepticus
Generalized tonic-clonic status epilepticus
Clonic status epilepticus
Absence status epilepticus
Tonic status epilepticus
Myoclonic status epilepticus

Focal status epilepticus
Epilepsia partialis continua of Kojevnikov
Aura continua
Limbic status epilepticus (psychomotor status)
Hemiconvulsive status with hemiparesis

TABLE 2 The International League Against Epilepsy (ILAE) classification of epilepsy syndromes and related conditions (2001)

Benign familial neonatal seizures
Early myoclonic encephalopathy
Ohtahara syndrome
* Migrating partial seizures of infancy
West syndrome
Benign myoclonic epilepsy in infancy
Benign familial infantile seizures
Benign infantile seizures (nonfamilial)
Dravet's syndrome
HH syndrome
* Myoclonic status in nonprogressive encephalopathies
Benign childhood epilepsy with centrotemporal spikes
Early onset benign childhood occipital epilepsy (Panayiotopoulos type)
Late onset childhood occipital epilepsy (Gastaut type)
Epilepsy with myoclonic absences
Epilepsy with myoclonic-astatic seizures
Lennox-Gastaut syndrome
Landau-Kleffner syndrome (LKS)
Epilepsy with continuous spike-and-waves during slow-wave sleep (other than LKS)
Childhood absence epilepsy
Progressive myoclonus epilepsies
Idiopathic generalized epilepsies with variable phenotypes
Juvenile absence epilepsy
Juvenile myoclonic epilepsy
Epilepsy with generalized tonic-clonic seizures only
Reflex epilepsies
Idiopathic photosensitive occipital lobe epilepsy
Other visual sensitive epilepsies
Primary reading epilepsy
Startle epilepsy
Autosomal dominant nocturnal frontal lobe epilepsy
Familial temporal lobe epilepsies
* Generalized epilepsies with febrile seizures plus
* Familial focal epilepsy with variable foci
Symptomatic (or probably symptomatic) focal epilepsies
Limbic epilepsies
Mesial temporal lobe epilepsy with hippocampal sclerosis
Mesial temporal lobe epilepsy defined by specific etiologies
Other types defined by location and etiology
Neocortical epilepsies
Rasmussen syndrome
Other types defined by location and etiology
Conditions with epileptic seizures that do not require a diagnosis of epilepsy
    Benign neonatal seizures
    Febrile seizures
    Reflex seizures
    Alcohol-withdrawal seizures
    Drug or other chemically induced seizures
    Immediate and early post-traumatic seizures
    Single seizures or isolated clusters of seizures
    Rarely repeated seizures (oligoepilepsy)

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* Syndromes in development

TABLE 3 Etiology of epilepsy

Inherited epileptic disorders
Acquired epileptic disorders
Hypoxic-ischemic encephalopathy
Head trauma
Cerebral stroke
Vascular malformations
Cortical dysplasias
Central nervous system infections
Brain tumors
Toxic central nervous system disturbances
Ammon's horn sclerosis

Epilepsia (2003) 44:S21-22

Epilepsies can generally be divided into three major categories based on etiology. Idiopathic epilepsies refer to contributing genetic factors in the development of seizures. In symptomatic epilepsies, there is a known (remote) etiologic lesion in the brain. The third category is probably symptomatic (previously called cryptogenic) in which the etiology is most likely symptomatic but the lesion cannot be identified (Engel 2001). The incidence of symptomatic epilepsy is highest in older age groups, especially over 65 years of age (Kotsopoulos et al. 2005), and its etiology is diverse. The most common etiologies for epilepsy are stroke, tumors, trauma and congenital disorders as well as dementia disorders (Forsgren et al. 1996; 2005) (Table 3). In acute symptomatic seizures alcohol or other substance withdrawal is the most common etiology (Forsgren et al. 1996).

Epilepsies are diagnosed on the basis of accurate description of the seizure, electroencephalography (EEG) (Figure 1), and structural neuroimaging. Epileptic syndrome, seizure type, and etiology largely determine which antiepileptic drug (AED) to choose in an individual patient. Response to the first AED is the most powerful predictor of long-term prognosis (Brodie and Kwan 2002). 60-70% of patients with

newly diagnosed epilepsy enter long-term remission, mostly on a single AED (Sillanpää et al. 1998; Kwan and Brodie 2000). Over 30% of patients have refractory epilepsy, i.e. they do not respond to adequate AED treatment (Kwan and Brodie 2000) and a subset of these patients develop progressive epilepsy (Collaborative Group for the Study of Epilepsy 1992). Prognosis is often thought to be poorer in patients with localization-related epilepsy, especially temporal lobe epilepsy (TLE), than with generalized epilepsies (Stephen et al. 2001).
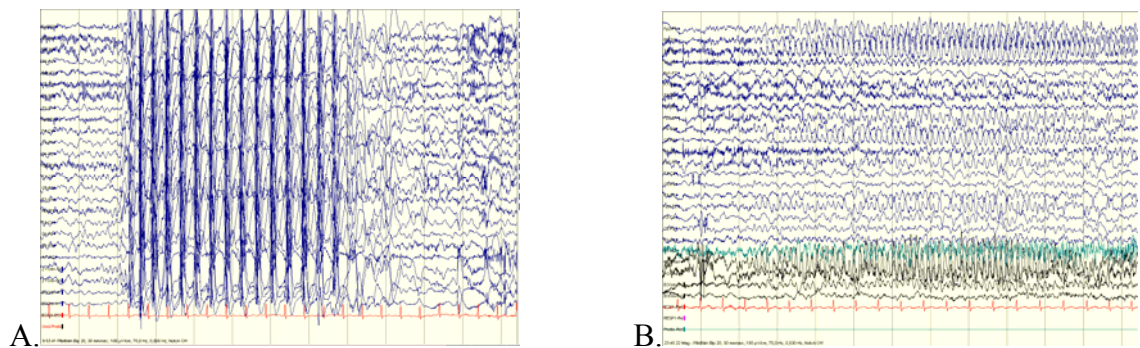
Figure 1. Ictal epileptic activity is visible in the EEG in the form of discharges in generalized absence epilepsy (A) and focal right temporal lobe onset epilepsy (B).

2. The effects of epileptic seizures

Both SE and recurrent seizures can have severe and lasting effects on the architecture of the brain. Some of the changes observed in experimental models or in clinical studies may be interpreted as either harmful or protective attempts to counterbalance the pro-epileptic effects (Parent et al. 2008). Several experimental animal models for SE and for epilepsies with focal seizures have been developed to gain understanding of the effects of seizures as well as the mechanisms involved in seizure-initiation, epileptogenesis, and to study treatment options. The most commonly used animal models are chemically

induced seizures by kainic acid, pentylenetetrazol (PTZ) and pilocarpine, or seizures induced by electrical stimulation either focally or by maximal electroconvulsive shock model. A common model of epileptogenesis is kindling, in which repetitive, subconvulsive electrical stimulation evokes progressively prolonged responses that culminate in generalized seizures (Pitkänen et al. 2007).

Epileptogenesis is the process that leads to the first spontaneous seizure after the primary, potentially seizure-causing event, e.g. SE, traumatic brain injury (TBI) or stroke. During this clinically silent latency period a number of parallel and sequential molecular and cellular events occur leading to increased excitability and development of recurrent spontaneous seizures (Figure 2). The latency period varies in length from weeks to years (Pitkänen et al. 2007).

2.1. Seizures and brain damage

Epileptic seizures can cause neuronal cell death, enhanced neurogenesis, axonal sprouting, dendritic changes, and reactive gliosis. It is difficult to assess the contribution of the underlying epileptogenic insult versus recurrent brief seizures to neuronal damage; thus controversy whether repeated brief seizures can cause neuronal damage continues (Pitkänen et al. 2002; Sutula, 2004). Histopathological analyses have suggested that both the initial insult and recurrent seizures contribute to the damage (Mathern et al. 1995). There is indication from the kindling model that brief recurrent seizures can lead to cell loss and sprouting (Cavazos et al. 1994; Sutula et al. 1996; Pitkänen and Sutula 2002).

PRIMARY BRAIN INJURY

Status epilepticus
Traumatic brain damage
Stroke
Cortical malformations
Perinatal injury
Cerebral infections

LATENCY PERIOD, EPILEPTOGENESIS

Secondary injury
-edema
-electrolyte imbalance
-excitotoxicity
-hypoxia, ischemia
-microthrombosis
-inflammation, expression of
cytokines and inflammatory
mediators

Reorganization
-neuronal loss
-glial cell death
-neurogenesis
-gliosis
-axonal injury
-plasticity
-angiogenesis

Altered excitability, neuronal death

EPILEPSY

Figure 2. Schematic representation of the factors that may be triggered by brain injury leading to epilepsy. Modified from Pitkänen and Sutula 2002.

Both experimental and clinical studies have shown that SE may induce neuronal damage (Young 2006). Activation of mesial temporal structures is more likely to cause damage than that of other areas of the brain (Holmes 2002); therefore, one of the consequences of prolonged seizures is selective neuronal loss in the hippocampus. There is histopathological, radiological and biochemical evidence that SE causes neuronal injury identical to mesial temporal sclerosis in humans and subsequently TLE (Fountain and Lothman 1995; Fountain and Freeman 2006). Typical neuronal necrosis in hippocampal areas CA1, CA3 and subiculum have been observed in autopsy specimens from patients who died during SE (DeGiorgio et al. 1992). Imaging studies have also reported progressive hippocampal atrophy and evolution of mesial temporal sclerosis after SE or prolonged seizures (Tien and Felsberg 1995; Wieshmann et al. 1997; Natsume et al. 2007).

2.2. Mechanisms of seizure-related neuronal injury

The damage caused by changes that are a direct consequence of seizure activity, i.e. excitotoxic damage, is considered the most important mechanism of injury during SE (Wasterlain et al. 1993; Fountain and Lothman 1995). Further, SE can cause brain damage by other physiological changes, i.e. hypoxia, and drug therapy of SE also carries risks. In seizures, there is a failure of gamma aminobutyric acid (GABA) −induced inhibition, and potentially neurotoxic increases in the extracellular levels of glutamate in the epileptogenic hippocampus have been demonstrated (Milgram et al. 1991; During and Spencer 1993). Changes in neuronal excitability induce abnormal activity in individual neurons and cause hyperexcitable cells to participate in synchronized

activities that occur through normal or pathological pathways (Avanzini and Franceschetti 2003). Recurrent seizures may trigger synaptic plasticity, modify molecular processes and lead to further facilitation of excitability and predisposition to seizures. This plasticity in neural circuits may induce progressive damage but it is also possible that it may provide resistance to additional damage (Sutula 2004). There is also evidence that programmed cell death contributes to neuronal damage in patients with epilepsy (Henshall et al. 2000).

Recent findings suggest that modified astroglial functioning may have a role in the generation and spread of seizure activity. Their dysfunction might be involved in the pathogenesis of epilepsy (Jabs et al. 2008; Schwarcz 2008). Further, immune and inflammatory reactions occur after seizures. Experimental studies in rodent models show that increased levels of inflammatory mediators have pro-ictogenic effects and can contribute to cell loss and blood-brain barrier (BBB) damage (Vezzani et al. 2008). Cytokines can also induce synthesis of mediators in brain repair, e.g. nerve growth factor, ciliary neurotrophic factor, and insulin-like growth factor from astrocytes (Allan and Rothwell 2001).

2.3. Clinical consequences of epileptic seizures

Several magnetic resonance imaging (MRI) studies have shown an association between severity of hippocampal damage and the estimated total seizure number, seizure frequency, and duration of epilepsy (Van Paesschen et al. 1997; Salmenperä et al. 2001). A prospective MRI study of patients with newly diagnosed focal epilepsy found that hippocampal damage occurs in individual patients in the course of 2 to 3 years of

follow-up. The patients who developed hippocampal volume decrease had seizures of longer duration and higher number before the diagnosis of epilepsy (Salmenperä et al. 2005). Neuropathological evidence from epilepsy surgery most commonly obtained from patients with refractory TLE shows that density and branching of dendritic spines are reduced (Babb and Brown 1986), there is sprouting of mossy fibers in the dentate gyrus (Proper et al. 2000), and astrocytosis is common (Doherty et al. 2007). Ongoing injury is suggested by the presence of activated microglia (Beach et al. 1995). However, not all patients with frequent seizures exhibit signs of damage, which underlines the heterogeneity of individual susceptibility (e.g. co-morbidities, genetic factors) to neuronal damage from severe epilepsy (Duncan 2002).

The risk of morbidity and mortality is highest in generalized tonic-clonic form of SE (GTCSE). Chronic encephalopathy and brain atrophy follow GTCSE in 6 to 15% of patients, presumably as a result of diffuse cortical injury (Dodrill and Wilensky 1990; Eriksson and Koivikko 1997). Often it is not possible to identify whether SE itself or the underlying clinical condition is the cause of the subsequent cognitive decline after SE (Helmstaedter 2007). GTCSE can have a negative effect on cognitive function, particularly memory (Dodrill 2002). However, the incidence of demonstrable cognitive deficits induced by SE in humans appears to be low (Dodrill and Wilensky 1990; Adachi et al. 2005). The development of future epilepsy is the most common identifiable long-term complication of GTCSE (Eriksson and Koivikko 1997; Hesdorffer et al. 1998). The risk of developing epilepsy after SE appears to be higher than after a single acute symptomatic seizure. In these cases, SE can be a marker for abnormal brain or damage caused by SE can contribute to subsequent epilepsy (Hesdorffer et al. 1998; Berg et al. 1996).

In rodents, both hippocampal lesions and experimental epilepsy reduce performance in cognitive tasks (Holmes 2002). Clinical and epidemiological studies have shown that a subset of patients with epilepsy has progressive features such as increasing seizure frequency and cognitive decline (Sutula 2004). Progressive symptoms are particularly common in TLE (Pitkänen et al. 2002). Neuropsychological and imaging evidence indicate that some patients have progressive cortical atrophy, and the effects of recurrent seizures on cognitive function have shown a slight but consistent relation between seizures and mental decline (Dodrill 2002; Helmstaedter 2002; Sutula and Pitkänen 2002). Furthermore, it is well established that children with epilepsy are known to have cognitive problems. Their general intellectual functioning is often lowered and specific cognitive impairments can occur (MacAllister and Schaffer 2007). Epileptic encephalopathies, such as Lennox-Gastaut or West Syndrome, are a group of syndromes that are characterized by regression of cognitive development or failure to reach developmental standards (Nabbout and Dulac 2003).

3. Electroconvulsive therapy (ECT) and depressive disorder

3.1. Depressive disorder

Depressive disorders are mental illnesses characterized by a profound and persistent feeling of depressed mood and/or a loss of interest or pleasure. Disturbances in sleep, appetite, and mental processes are common symptoms. To qualify for diagnostic criteria these symptoms should cause clinically significant distress or impairment in social, occupational, or other important areas of functioning (American Psychiatric Association

1994). A study by the World Health Organization ranked depression the fourth common global burden of disease and found it to be the largest non-fatal one (Ustün et al. 2004). Major depressive disorder (MDD) has an estimated lifetime prevalence of about 17 % (Kessler et al. 2005). Although the pathogenesis of MDD is complex and not fully understood, depression affects integrated neural pathways shared by monoaminergic neurotransmitter systems (Delgado 2000). There is also growing evidence that neurotrophic mechanisms are important pathogenetic factors in depression as well as in the action of antidepressant treatment (Gould et al. 2003). Treatments for depression include psychological interventions, pharmacological treatment and ECT for medication resistant severe depression (American Psychiatric Association 2000).

3.2. ECT

ECT is the most effective treatment for patients with severe depression, with medication resistance its leading indication (The UK ECT Review Group 2003). The efficacy of ECT has also been well documented in mania and some forms of schizophrenia (American Psychiatric Association 2001). Use of ECT was introduced when it was realized that symptoms of schizophrenia were reduced after spontaneous epileptic seizures (Meduna 1936; Fink 1984). ECT is generally considered a safe treatment and in more recent decades its use has included muscle-relaxants, general anesthesia and oxygenation. However, the current use of ECT is relatively infrequent partly because of its limited availability (Huuhka 2005). An adequate generalized epileptic seizure is necessary for a therapeutic response (Beyer et al. 1998). The neurobiological action of ECT is not fully understood, but it is known that ECT has effects on several

neurotransmitters and their receptors, neuropeptides, hormones and neurotrophic factors (Wahlund and von Rosen 2003). Neurogenesis, neurite outgrowth, and neuronal plasticity associated with brain-derived neurotrophic factor (BDNF), glutamate, and cAMP-protein kinase A signalling pathways may mediate the antidepressant effects of ECT (Altar et al. 2004).

3.3. Neuronal damage after ECT

Although ECT is considered safe, the most common adverse effects associated with ECT are transient memory loss and related cognitive dysfunction. ECT may induce acute postictal disorientation and anterograde or retrograde amnesia (Calev et al. 1991; Sackeim et al. 1993; Rose et al. 2003). These adverse effects are largely reversible and short-lived, although cognitive side effects have been detected even after 6 months after ECT (Sackeim et al. 2007). Electrical waveform and electrode placement correlate with the risk of cognitive side effects, e.g. bilateral ECT results in more severe and persisting retrograde amnesia than right unilateral ECT (Sackeim et al. 2007). Advancing age and lower premorbid intellectual function have been associated with greater cognitive deficits suggesting that patients with greater premorbid abilities can better compensate for the impact of ECT on cognitive functions (Sackeim et al. 2007). Persistent cognitive adverse effects observed in selected patients have raised a concern that ECT may induce neuronal damage. However, no evidence of structural brain damage due to ECT has been found in structural brain imaging studies, autopsy reports of patients who had received ECT or animal studies of electroconvulsive shocks (ECS) (Devanand et al. 1994).

4. Markers of seizure-related brain damage

Markers of brain damage could help determine the critical duration of epileptic seizures prior to neuronal injury, define subtypes of epilepsy requiring more aggressive interventions and possibly serve as a prognostic tool. Various biomarkers have been studied in the context of epilepsy and brain damage, e.g. BDNF (Toro et al. 2007), myelin basic protein (MBP), glial fibrillary acidic protein (GFAP) (Gurnett et al. 2003), neurofilament (Lamers et al. 2003), and different enzymes (enolase, aldolase, pyruvate kinase, lactate dehydrogenase, creatine phosphokinase) (Royds et al. 1983). However, neuron-specific enolase and S-100b protein are the most widely investigated biochemical markers of nervous tissue damage, present only in low concentrations outside the nervous system (Kato et al. 1982).

4.1. Neuron-specific enolase (NSE)

NSE is a γγ-isoenzyme of the glycolytic enzyme enolase involved in the glycolysis pathway at the conversion of 2-phosphoglycerate to phosphoenolpyruvate. NSE originates predominantly from the cytoplasm of neurons and neuroendocrine cells. Neuronal damage and impairment of the BBB integrity can be detected by the release of NSE into cerebrospinal fluid (CSF) and eventually into the blood (Schmechel et al. 1978) NSE is therefore regarded as a marker of neuronal damage and prognosis in various neurological disorders associated with cell damage in the central or peripheral nervous system. The serum levels of NSE increase also in neuroendocrine tumors, such as small-cell lung cancer and neuroblastoma. NSE is used as a tumor marker for lung

cancers and has been suggested as a useful marker in monitoring the response to therapy and in the detection of early recurrences (Karnak et al. 2005).

4.1.1. NSE and brain damage

Elevated levels of NSE in either CSF or serum have been found in neurological conditions such as TBI, focal or global ischemia, hemorrhagic brain damage, SE, and Guillain-Barré syndrome (Roine et al. 1989; Vermuyten et al. 1990; Vos et al. 2004; Anand and Stead 2005; Zandbergen et al. 2006). Although increased levels of CSF NSE have been measured in patients with Creutzfeldt-Jakob disease (CJD) or different types of dementias in some studies (Wakayama et al. 1987; Blennow et al. 1994; Zerr et al. 1995), the measurement of CSF NSE has not been additionally helpful in differentiating CJD or Alzheimer's disease (AD) from other neurodegenerative disorders (Parnetti et al. 1995; Bahl et al. 2008).

Serum NSE has been more widely used in assessing the magnitude of primary damage as well as in predicting outcome after hypoxic (Böttiger et al. 2001; Rech et al. 2006) and TBI (Vos et al. 2004) and ischemic stroke (Wunderlich et al. 1999). Levels of CSF and serum NSE correlated with the prognosis of hypoxic brain damage after cardiac arrest (Roine et al. 1989; Pfeifer et al. 2005), and treatment with hypothermia was associated with declining levels of serum NSE, as compared with normothermia-treated patients (Tiainen et al. 2003). Serum NSE levels rose twofold after severe TBI compared to normal values (Vos et al. 2004) and these levels in serum or CSF have been found to correlate with the outcome in several studies (Dauberschmidt et al. 1983; Herrman et al.

1999;2001; Vos et al. 2004). High serum NSE levels have usually indicated worse outcome in patients with ischemic stroke, and they correlated with volume of infarcted tissue (Anand and Stead 2005), although contradictory results have also been reported (Cunningham et al. 1991; Missler et al. 1997).

The magnitude of clinically significant increase in NSE levels in respect of brain damage is not well established. The upper normal levels for NSE also vary in previous studies because of different determination methods. It is therefore recommended that each laboratory should obtain its own reference values (Casmiro et al. 2005). In a normal population CSF and serum NSE concentrations were 17.3 ± 4.6 and 8.7 ± 3.9 µg/L, respectively. Using serum NSE with a cut-off point of > 80 mg/L for predicting persisting coma after cardiopulmonary resuscitation, specificity was 100 % with a sensitivity of 65 % (Reisinger et al. 2008). Sex or age does not affect the levels of NSE (Casmiro et al. 2005). Hemolysis produces false elevated values of NSE, and hemolysed samples should not be included for analysis.

4.1.2. NSE and epileptic seizures

Evidence obtained by animal models has shown that markedly elevated CSF or serum NSE levels are associated with irreversible neuronal injury following SE and complex partial status epilepticus (CPSE) as confirmed by histological data (Sankar et al. 1997; Schreiber et al. 1999; Hasegawa et al. 2002). In humans CSF and serum NSE levels obtained within the first 24 or 48 hours were elevated and correlated well with the duration of SE and outcome of patients (DeGiorgio et al. 1995; Correale et al. 1998).

Only a few studies have shown elevated NSE levels also in CPSE and non-convulsive status epilepticus in humans (DeGiorgio et al. 1996; O'Regan and Brown 1998; DeGiorgio et al. 1999).

The studies reporting levels of either CSF or serum NSE shortly after single, brief seizures are controversial. In rat brain, CSF levels of NSE presented a biphasic increase after brief single tonic-clonic seizure induced by pentylenetetrazol (Oses et al. 2004). Several studies have found elevated NSE levels, but only in a subset of patients with no identifiable factors associated with elevations. Elevated CSF NSE levels have been reported in 3 out of 10 patients with epilepsy measured a few days postictum (Jacobi and Reiber 1988). CSF NSE was also increased after methohexital-induced electrographic seizures in all three patients undergoing epilepsy surgery (Rabinowicz et al. 1994). Elevated levels of serum NSE have been observed after single tonic-clonic seizures (Greffe et al. 1996; Büttner et al. 1999) and during inpatient video/EEG monitoring (Rabinowicz et al. 1996; Tumani et al. 1999; Willert et al. 2004). The studied seizure type has mainly been generalized tonic-clonic (GTCS) seizure. Only a few analyses have been done after partial seizures, after which NSE can also increase, although a more pronounced increase has been seen after secondary generalized seizures (Pitkänen and Sutula 2002). Furthermore, cisternal CSF levels of NSE in TLE patients were more elevated on the side of seizure origin (Steinhoff et al. 1999). CSF and serum NSE levels were normal in children with febrile seizures (Rodrígues-Núñez et al. 2000; Borusiak and Herbold 2003), although an association between the duration of partial febrile seizures and CSF NSE levels has been observed (Tanabe et al. 2001).

Previous ECT studies using variable methods (e.g. varying ECT technology, sampling only during the first ECT session or after repeated ECTs) have shown no systematic serum NSE changes after ECT-induced seizures (Berrouschot et al. 1996; Greffe et al. 1996; Agelink et al. 2001). Only three patients showed a transient increase in the levels of NSE after ECT (Greffe et al. 1996; Agelink et al. 2001). No correlation with the ECT features (energy doses, seizure durations, cognitive performance tests) was observed.

4.2. S-100b protein (S-100b)

S-100 is a mixture of dimeric proteins consisting of two subunits α and β. S-100b (ββ-S-100) is present in high concentrations in glial cells and Schwann cells. S-100b protein (S-100b) is a calcium-binding protein, which has many intracellular and extracellular functions in the central nervous system (CNS) (Donato 2001). Under normal circumstances, S-100b promotes the growth of neurites, stimulates astrocyte proliferation, and increases free calcium concentrations in both neurons and astrocytes (Zimmer et al. 1995; Donato 2001). Increase in S-100b may reflect either glial damage or astrocytic reactions to neural injury, and these reactions may have neuroprotective properties (Herrmann et al. 2000; Pleines et al. 2001).

4.2.1. S-100b as a marker of brain damage

The levels of S-100b in serum and CSF are elevated after different types of brain damage such as focal and global ischemia (Martens et al. 1998; Pfeifer et al. 2005; Nash

et al. 2008), TBI (Kleindienst et al. 2007), bacterial meningitis (Lins et al. 2005) and subarachnoid hemorrhage (Wiesmann et al. 1997). S-100b seems to be quite an ideal marker for acute brain ischemia (Nash et al. 2008). After ischemic stroke the CSF and serum levels of S-100b are significantly increased when measured within 0-180 h after the onset of symptoms. The peak values have been found at 24-120 h (Nash et al. 2008). Higher serum S-100b values indicate larger infarction volumes, more severe strokes and the levels correlate with an outcome of stroke patients (Abraha et al. 1997; Missler et al. 1997; Foerch et al. 2005). Elevated S-100b levels in serum were also a risk factor for hemorrhagic transformation after thrombolytic therapy in patients with acute stroke (Foerch et al. 2007). S-100b is being used as a prognostic marker after global hypoxic-ischaemic brain damage (Rosén et al. 1998; Böttiger et al. 2001; Pfeifer et al. 2005). Especially, daily serum measurements have been reported as useful in estimating the outcome of these patients (Pfeifer et al. 2005). In trauma patients extracerebral origin of S-100b must be taken into account, because damaged skeletal muscle or adipose tissue can contribute to elevated levels (Bloomfield et al. 2007; Kleindienst et al. 2007). However, a correlation between serum S-100b levels and severity of the damage and outcome in severe head injury has been demonstrated in several studies (Hayakata et al. 2004; Savola et al. 2004; Townend et al. 2006a). Even more attempts have been made to prove correlation between serum S-100b levels and minor head trauma. So far, normal S-100b levels cannot be used to exclude patients from computed tomography (CT) scanning, but elevated levels support selection of patients for further imaging (Müller et al. 2007).

The levels of serum S-100b in healthy controls have been observed to fall between 0.018 and 0.098 µg/L (Arts et al. 2006; Müller et al. 2006). The magnitude of increase in

serum S-100b levels seen in overt brain damage (e.g. head trauma and ischemic injury) varies (Bloomfield et al. 2007), but at least ten-fold increases have been noted in moderate to severe brain injury (Savola et al. 2004). Many studies have used a cut-off level of 2.0-2.5 µg/L in patients with TBI (Townend et al. 2006a). For example, with S-100b levels of ≥ 0.2 µg/L at day two after cardiac arrest the positive predictive value for fatal outcome was 100 % and the negative predictive value was 89 % for survival (Rosén et al. 1998). At any rate, sustained elevations of S-100b in serum over 24 hours can more reliably predict the extent of brain injury (Bloomfield et al. 2007). The half-life of S-100b is short, 20-25 minutes (Jönsson et al. 2000; Townend et al. 2006b), thus possible early and transient increases may not become detected depending on study protocols. Whether the S-100b concentration is age dependent is controversial (Nygaard et al. 1997; Portela et al. 2002). Some studies have shown age dependency but others have not. For example, Portela et al. (2002) found a negative correlation between S-100b and age in the first 20 years but not after the age of 20.

4.2.2. S-100b as a marker of glial activation

S-100b can regulate cell-cell communication, cell growth and cell structure, energy metabolism, contraction and intracellular signal transduction (Zimmer et al. 1995; Donato 2003). Extracellular S-100b has a dual effect on neurons depending on its concentration. At nanomolar concentrations it has neurotrophic and neuroprotective effects, whereas at micromolar doses it can cause neuronal death via apoptosis (Donato 2001). It may play a role in synaptic plasticity in memory and learning (Whitaker-Azmitia and Azmitia 1994) or, in higher concentrations, S-100b may impair spatial

learning (Gerlai and Roder 1996) and cause cell death (Whitaker-Azmitia et al. 1997). 5-
HT1A receptor agonists, glutamate, adenosine, and lysophosphatydic acid stimulate
release of S-100b by astrocytes. The dual effect of S-100b has raised questions of
whether its injury-induced increase is due to a passive release of astrocytes or an active
release by stimulated astrocytes initiating repair mechanisms (Kleindienst et al. 2007;
Sen and Belli 2007). Furthermore, the therapeutic effects of S-100b have been implied in
experimental trauma models (Kleindienst et al. 2004; 2005).

4.2.3. S-100b and epileptic seizures

Only three previous studies have measured S-100b levels in patients shortly after
seizures. In six epileptic children the serum levels of S-100b after the seizure were 2-3
times higher than in the control group (Sendrowski et al. 2004). Two other studies
reporting the serum levels of S-100b in adult patients after single tonic-clonic seizures
observed no postictal elevation (Büttner et al. 1999; Leutmezer et al. 2002). Serum S-
100b levels were normal also interictally in patients with focal epilepsy (Portela et al.
2003) but cisternal CSF S-100b was elevated and site-specific in intractable TLE
patients (Steinhoff et al. 1999). CSF S-100b levels increased early in rat after seizure
induced by PTZ, possibly indicating an astrocytic reaction aiming to promote neuronal
stability and neuroprotection (Oses et al. 2004). Furthermore, S-100b knockout mice
showed more susceptibility to seizures than wild-type mice in a kindling model (Dyck et
al. 2002).

Increased CSF or serum levels of S-100b are not commonly observed after ECT (Zachrisson et al. 2000; Agelink et al. 2001). Only one previous study found a small but significant rise in S-100b levels at 1 hour, with similar but reduced effect at 3 hours after ECT (Arts et al. 2006). Patients with higher S-100b levels pre-ECT were also more likely to display poorer working memory but less subjective cognitive impairment post-ECT and less depression at follow-up.

4.2.4. S-100b and mood disorders

Evidence has suggested that neurodegeneration might be a pathogenetic factor in the development of major depression and schizophrenia (Rothermundt et al. 2003). In patients with psychiatric disorders, the levels of serum S-100b have been elevated, especially with mood disorders, bipolar disorder during episodes of mania and depression, and schizophrenia (Wiesmann et al. 1999; Machado-Vieira et al. 2002; Schroeter et al. 2002; Arolt et al. 2003; Andreassa et al. 2007). Serum S-100b levels may be reduced as a response to antidepressant treatment, suggesting its neuroprotective function (Schroeter et al. 2002; Arolt et al. 2003). However, it remains unclear whether an increase in serum S-100b in these conditions is due to destruction of astrocytes or to an active release of S-100b from intact astrocytes attempting to repair neuronal damage (Dietrich et al. 2004).

4.3. Tau and phosphorylated tau protein

Tau is a microtubule-associated protein with a major role in normal microtubular function in axons. It promotes the polymerization of tubulin into microtubules and stabilizes microtubules. Phosphorylation of tau protein decreases its ability to promote microtubular assembly. Under normal conditions tau protein is mildly phosphorylated and the equilibrium between phosphorylation and dephosphorylation modulates cytoskeletal stability and axonal morphology (Mandelkow et al. 1995). AD and many other human neurodegenerative conditions are characterized by hyperphosphorylation of tau (Lace 2007). CSF total tau (T-tau) and especially phosphorylated tau (P-tau) measurements have been used in the diagnosis of AD. Hyperphosphorylation of tau is thought to lead to neurofibrillary changes, a neuropathological hallmark of AD, and P-tau in CSF correlates with neocortical neurofibrillary pathology in AD (Buerger et al. 2006).

Tau protein is primarily localized in neuronal axons, and after brain parenchymal damage its release into CSF may increase (Delacourte 1994). Increased CSF T-tau levels have been found in patients with severe TBI (Franz et al. 2003; Öst et al. 2006), acute ischemic stroke (Hesse et al. 2000), intracerebral hemorrhage, multiple sclerosis and viral encephalitis, thus suggesting that t-tau CSF levels reflect the extent of axonal damage and neuronal degeneration (Süssmuth et al. 2001). CSF tau levels are very high in patients with CJD (Riemenschneider et al. 2003). Only one case report based on a single patient has reported a transient elevation of CSF tau measured four days after the

seizure (Matsui et al. 2007). Other than that, tau levels have not previously been measured after epileptic seizures.

AIMS OF THE STUDY

In these series of studies the purpose was to determine the degree and nature of possible brain damage after epileptic seizures. The established markers of brain damage, NSE, S-100b and tau were used in different patient groups with seizures. The specific aims were

i to search for evidence of neuronal damage after previously undiagnosed and untreated single tonic–clonic seizures;

ii to search for evidence of neuronal damage and to study temporal association of changes in biomarkers in patients with chronic refractory localization-related (focal) epilepsy;

iii to evaluate the association of increased tau levels with tonic–clonic seizures due to various etiologies; and

iv to investigate neuronal damage or glial activation in patients treated with ECT.

SUBJECTS AND METHODS

The patients and control subjects included in this study were treated at the Departments of Neurology (I, II, III) and Psychiatry (IV), Tampere University Hospital, Finland. All patients were fully informed of the risks and potential benefits of the CSF examination. The patients and control subjects gave their written informed consent. The study protocol was approved by the Ethics Committee of the Tampere University Hospital.

1. Patients

1.1. Patients with newly onset seizures (I)

A total of 22 consecutive patients (mean age 39 years, range 15–60) with single, previously undiagnosed and untreated tonic–clonic or partial secondarily generalized seizures were included in the study. The CSF samples were taken within 24 h (mean 15.1 h) after the seizure. Patients with seizures associated with electrolyte disturbances, metabolic causes, acute brain disease or trauma were excluded. 12 patients had single seizures; six patients had another seizure in emergency room. Six patients had seizures due to alcohol withdrawal. The duration of seizures was 1 to 15 min according to inpatient records. After the first epileptic seizure patients usually underwent EEG and CT or MRI examinations. There were two cerebral tumors (meningeoma and glioblastoma multiforme) in the epileptic group. The other patients had normal CT/MRI findings.

1.2. Refractory epilepsy patients with repeated seizures (II)

We included 31 patients with chronic refractory, partial epilepsy, 16 women and 15 men, with a mean age of 34 years (range 16-58 years). The patients were admitted for 24-h inpatient video-EEG monitoring to characterize and localize seizures or adjust medication. Baseline (0 h) serum samples were collected when the patients were admitted for video-EEG monitoring, and at 3 h, 6 h, 12 h, and 24 h after index seizure. The first clearly identifiable ictal event was selected as index seizure. Seizure burden was measured by total duration of seizures recorded during the 24-hour sampling period. The epilepsy syndrome was established according to ILAE diagnostic criteria. The patients were categorized into TLE and extratemporal epilepsies (XTLE) based on video-EEG recordings and MRI findings, as was the etiology of epilepsy (Table 4). There were six patients on monotherapy, 24 on polytherapy (mean number of medications 2.6), one had no medication, and three patients were treated with vagus nerve stimulation (Table 5).

TABLE 4 Characteristics of the patients with refractory epilepsy

Epilepsy syndrome	No. of patients	Male / Female	Age, mean (range)	MRI findings			
				Normal	HS	Cortical dysplasia	Other
TLE	15	8 / 7	40 (20-58)	4	9	1	1
XTLE	16	7 / 9	28 (16-52)	8	-	4	4

TLE, temporal lobe epilepsy
XTLE, extratemporal epilepsy

TABLE 5 Information on epilepsy of the patients with refractory epilepsy

Epilepsy syndrome	Mean duration of epilepsy, years (range)	Mean seizure frequency, per month* (range)	Index seizure type			Patients on mono- / polytherapy	Number of seizures in 24 h, mean (range)	Mean seizure burden, seconds (±S.D.)
			SPS	CPS	SGTCS			
TLE	24.2 (2-56)	7.4 (0.5-26)	1	11	3	3 / 12	2.6 (1-11)	259 (216)
XTLE	16.2 (1-52)	40.8 (0.5-200)	4	10	2	3 / 12	4.7** (1-12)	2492 (7704)

TLE, temporal lobe epilepsy; XTLE, extratemporal epilepsy; HS, hippocampal sclerosis; SPS, simple partial seizure; CPS, complex partial seizure; SGTCS, secondary generalized tonic-clonic seizure; *during the last year; ** two patients with 100 and 200 seizures (during the 24 h) excluded

1.3. Patients with acute seizures (III)

A total of 54 patients (mean age 48 years, range 16-88 years) with tonic–clonic or partial secondarily generalized seizures were included in the study. The patients had either single or recurrent seizures (median number of seizures 1.5). Nine patients had SE. The CSF samples were taken within 48 h (mean 14.4 h) after the seizure. The patients were divided into groups based on the underlying etiology of epileptic seizures. The seizures were caused by alcohol withdrawal in group 1 (ALCO). In groups 2 and 3 the patients had a diagnosis of epilepsy. Group 2 (SYMPT) had remote symptomatic epilepsy and in group 3 (EPI) the patients had either cryptogenic focal (n=10) or idiopathic epilepsy (n=3) with no related findings in brain CT or MRI. In group 4 (ACU) seizures were caused by acute systemic illness (sepsis, hyponatremia) or acute CNS disorder (acute

brain infarction, encephalitis, necrotizing encephalomyelitis). In addition to these four

groups, three patients had only a single seizure of unknown etiology (Table 6).

TABLE 6 The patients divided into four etiologic groups

	Group 1 (ALCO)	Group 2 (SYMPT)	Group 3 (EPI)	Group 4 (ACU)	Controls
No. of patients	15	16	13	7	31
The mean age (range)	50 (31-69)	60 (25-88)	36 (16-73)	45 (16-75)	40 (15-56)

Group 1 (ALCO), alcohol withdrawal
Group 2 (SYMPT), remote symptomatic epilepsy
Group 3 (EPI), cryptogenic focal, or idiopathic epilepsy
Group 4 (ACU), acute systemic or CNS illness

1.4. Patients with depression (IV)

Ten patients scheduled for treatment with ECT (mean age 56 years, range 28-70 years)

were included in the study. The serum samples were collected before ECT and at 1 h, 2

h, 6 h, 24 h, and 48 h after the treatment. All the patients were diagnosed with MDD;

four of them showed psychotic features. The patients were otherwise healthy except for

one patient (no. 7) who had ischemic heart disease and a history of a stroke 6 months

before the treatment. The patients' psychotrophic medications were continued

unchanged during the treatment. The severity of depression was scored with

Montgomery-Åsberg Depression Rating Scale (MADRS) and Beck Depression

Inventory (BDI) before and after the series of ECT. Mini Mental State Examination

(MMSE) scores were also assessed before and after ECT (Table 7).

TABLE 7 Characteristics of the patients with depression

Patient No.	Age/ Sex	Medication group	BDI Pre/post- ECT	MARDS Pre/post- ECT	MMSE Pre/post- ECT	No of ECT*
1	70/F	1,3	25/0	25/0	30/30	7
2	64/M	1,3	27/2	39/1	27/29	1
3	33/F	2,3	34/5	29/3	-/30	1
4	73/F	1,2,3	31/7	32/9	27/27	1
5	57/F	1,2	22/19	18/6	25/24	5
6	28/F	1,2	34/1	31/11	30/27	4
7	62/F	1,3	55/32	34/9	29/30	3
8	62/F	1,2	13/0	21/7	28/24	5
9	41/M	2	28/38	29/33	29/29	6
10	66/M	1,2,3	36/17	32/10	25/21	5
Mean	56		31/12	29/9	28/27	

Medication group: 1, neuroleptics; 2, antidepressants; 3, benzodiazepines
BDI, Beck Depression Inventory (maximum 63)
MARDS, Montgomery-Åsberg Depression Rating Scale (maximum 60)
MMSE, Mini-Mental State Examination (maximum 30)
ECT, electroconvulsive therapy

* Out of succession of treatments

2. Control groups

In studies I and III, the control samples were obtained from 20 (I) and 31 (II) adult patients with neurological symptoms (e.g. dizziness, headache) on whom lumbar puncture (LP) was performed to exclude neurological disease. Clinical examination or imaging studies (CT or MRI of the head) revealed no pathological findings. The mean age in the control groups was 40 years (16–56 years) (I), and 40 years (range 15-56 years) (II).

3. Methods

3.1. Processing of the samples

The first 2 ml of CSF was used for routine examination and a further 200 μl for the studies. Blood was collected within 30 min of LP in a Vacutainer EDTA vacuum tube and centrifuged at 3000 rpm for 10 min. Hemolyzed samples were not included for NSE analysis. The serum and CSF samples were stored at −70°C prior to analysis.

3.2. NSE

NSE assays were performed using an enzyme immunoassay technique (Cobas Core® NSE EIA, Hoffmann-La Roche, Switzerland) (I, IV) and electrochemiluminescense immunoassay technique (ECLIA) (Elecsys® 2010 Immunoassay Analyzer, Roche Diagnostics GmbH) (II). The lower detection limit for NSE was < 0.05 μg/L. The assays were performed according to manufacturers' protocol. The upper limit used for normal values of NSE was 17 μg/L by Roche's instructions.

3.3. S-100b

The S-100b concentrations were measured by an immunoluminometric assay for the quantification of protein (LIA-mat® Sangtec®100, Sangtec Medical, Sweden) (I, IV)

and electrochemiluminescense immunoassay technique (ECLIA) (Elecsys® 2010 Immunoassay Analyzer, Roche Diagnostics GmbH) (III). The sensitivity of the S-100b assay was <0.02 μg/l (I, IV) and < 0.005 μg/L (II). The assays were performed according to manufacturers' protocol. The upper limit used for normal values of S-100b was 0.11 μg/L by Roche's instructions.

3.4. T-tau and P-tau

The CSF levels of T-tau and P-tau$_{(181P)}$ were measured by a commercial enzyme-linked immunosorbent assay, ELISA (Innogenetics, Ghent, Belgium) according to the manufacturer's protocol. The ELISA analyses were done blinded to the diagnostic group. Two patients in group 4 (ACU) and two in controls did not have enough CSF for P-tau measurement. P-tau/T-tau ratio was also calculated. A concentration of T-tau above 400 pg/mL for patients over 60 years was considered abnormal as established previously (Herukka et al. 2005). We used 300 pg/mL as reference value for patients below 60 years, as previous studies have suggested lower reference values for younger patients (Sjögren et al. 2001).

3.5. ECT procedure

All patients were treated with bilateral ECT administered with a Thymatron DGx (Somatics Inc, Lake Bluff, Ill) brief-pulse device. The initial-stimulus dosage (millicoulombs) was adjusted to all patients with the age method, being about five times

their age (Swartz and Abrams 1996). Anesthesia was induced with propofol (5/10 patients) or methohexital (5/10 patients) and muscle relaxation with succinylcholine. The arterial oxygen saturation, heart rate and three-lead ECG were continuously monitored. The seizure duration was measured with EEG and convulsive motor response with electromyogram (EMG). All patients experienced an adequate electrical generalized seizure (mean seizure duration 45.7 seconds) and the mean energy used was 272.2 mC.

3.6. Statistical methods

Mean and standard deviations were calculated for variables and medians and quartiles for non-parametric variables. Pearson's correlation (parametric) and Spearman's correlation (non-parametric) were used to asses associations between variables. Statistical significance of differences between two groups was tested by independent two-tailed t-test in study I. Friedman analysis of variance (ANOVA) with post hoc comparisons (Wilcoxon matched pairs test), Kruskall-Wallis ANOVA and Mann-Whitney U-test were used to compare concentrations of NSE and S-100 and T-tau and P-tau in studies II, III and IV when appropriate. Area under curve (AUC) values were estimated using MedCalc (ver. Win 9.1.0.1, MedCalc software, Mariakerke, Belgium) (Matthews et al., 1990). Frequency tables were compared with Pearson chi-square test (in 2 x 5 tables, 4 degrees of freedom) and Fisher's exact test (post-hoc comparisons in 2 x 2 tables, 1 degree of freedom). Statistical calculations were carried out using Statistica (ver. Win 5.1D, Statsoft Inc., Tulsa, OK, USA). Findings were considered statistically significant at P values less than 0.05.

RESULTS

The following results of biomarkers after epileptic seizures were obtained from the patients with newly onset seizures, refractory epilepsy patients with repeated seizures; and patients with depression undergoing ECT treatment. CSF tau measurements were also evaluated in patients with epileptic seizures due to different etiologic groups.

1. CSF and serum levels of NSE

There were no significant differences in the mean CSF and serum NSE levels between the patients with newly onset seizures and the control group (I, Table 8) or in the median serum NSE levels in depressed patients after ECT at various time points (IV, Table 9). Only three patients in the epilepsy group had CSF levels of NSE over 12 μg/l (Table 10). The two patients in the epilepsy group who were found to have tumors (meningeoma and glioblastoma multiforme) also had normal values (CSF NSE 3 and 9 μg/l, respectively). There was no correlation between serum and CSF levels of NSE (r = 0.13, $P = 0.40$)

In the patients with refractory epilepsy the serum levels of NSE in the TLE group showed a statistically significant increase, whereas in the XTLE group the changes were not significant (II, Table 9). As the increase in the NSE concentration after an index seizure seemed to be single peaked and linear, we calculated the AUC curves for each subject (Matthews et al., 1990). The difference in NSE AUCs from the baseline (0 h) to the 24 h end-point was

clearly significant ($AUC_{0\text{-}24\,h}$ for TLE 78.64 ± 48.22 vs. XTLE 24.58 ± 35.89, *P* < 0.005). This difference stands for net NSE concentration difference in TLE and XTLE during the whole video-EEG monitoring. The major difference was caused by the marked increase in NSE in TLE from 6 to 12 hours as well as 12 to 24 hours postictal time intervals ($AUC_{6\text{-}12\,h}$ for TLE 25.6 ± 20.6 vs. XTLE 7.8 ± 13.37, *P* < 0.013 and $AUC_{12\text{-}24\,h}$ for TLE 36.67 ± 28.53 vs. XTLE 6.69 ± 19.10, *P* < 0.009). NSE levels in TLE started to increase at 3 h and the time to reach maximum was 10.2 ± 7.76 h for TLE and 7.88 ± 5.78 h for XTLE after index seizure (*P* = 0.39). Five patients had only one brief seizure. The number of seizures or seizure burden did not correlate with the levels of NSE. Also five patients had a secondary generalized tonic-clonic seizure (SGTCS) as index seizure but SGTCS did not explain the increases of NSE. The mean levels of NSE were not significantly increased either within or between the subgroups of patients based on different etiologies of epilepsy. Furthermore, the increases of NSE did not correlate with the duration of epilepsy.

TABLE 8 CSF and serum levels of neuron-specific enolase (NSE) in patients with newly onset seizures and controls (I)

NSE μg/l	Patient group (n = 22)	Control group (n = 20)	*P* value
CSF (mean ± S.D.)	8.9 (6.9)	13.1 (6.1)	0.60
Serum (mean ± S.D.)	8.2 (2.8)	8.0 (2.1)	0.21

TABLE 9 Serial serum neuron-specific enolase (NSE) in temporal lobe epilepsy (TLE) and extratemporal epilepsy (XTLE) patients (II) and depressed patients treated with electroconvulsive therapy (ECT) (IV)

NSE µg/l	0 h	1 h	2 h	3 h	6 h	12 h	24 h	48 h	P value
TLE (n = 15)									
mean	8.4			11.4*	13.5*	13.0*	10.3*		0.006
(± S.D.)	(2.6)			(3.8)	(4.5)	(5.5)	(3.1)		
XTLE (n = 16)									
mean	9.4			11.3	10.3	10.1	9.4		0.30
(± S.D.)	(1.5)			(3.5)	(3.4)	(2.8)	(2.7)		
ECT (n = 10)									
median	7.0	8.0	8.0		7.0		7.5	8.5	0.30
(quartiles)	5.8-9.3	6.8-10.0	8.0-9.0		7.0-9.0		5.0-9.0	6.8-11.3	

* Wilcoxon matched pairs; 0 h < 3 h, $p = 0.005$; 0 h < 6 h, $p = 0.0009$; 0 h < 12 h, $P = 0.002$; 0 h < 24 h, $P = 0.008$

TABLE 10 Characteristics of three patients with elevated CSF neuron-specific enolase (NSE) (I)

Age / sex	CSF NSE µg/l	Serum NSE µg/l	Seizure	Imaging
56 / M	19	13	1 short uncomplicated	Normal
60 / F	28	10	2 (1 prolonged)	Atrophy
41 / M	23	5	2 short	Normal

2. CSF and serum levels of S-100b

There were no significant differences in the mean CSF and serum S-100b levels between the patients with newly onset seizures and control subjects (I, Table 11). In refractory epilepsy patients with repeated seizures the serum levels of S-100b in the TLE group showed a statistically significant increase, but shorter and smaller than the levels of NSE. Again there were no changes in the XTLE group (II, Table 12). In the patients treated with ECT (IV), there was a trend towards an increase in S-100b levels at 2h. The changes were, however, not statistically significant (Table 12). In four patients the S-100b levels were increased at least 2-fold at 2 h compared to the baseline, the maximum elevation being 8-fold. The changes in the levels of S-100b did not correlate with the parameters of the treatment, e.g. seizure duration, energy used or the number of ECT session. The pre-to-post ECT changes in MADRS and BDI scores correlated with the concentration of S-100b at 2 h (MADRS, $r = 0.68$, $P = 0.044$; BDI, $r = 0.65$, $P = 0.60$) and at 6 h (MADRS, $r = 0.73$, $P = 0.040$; BDI, $r = 0.77$, $P = 0.027$). In addition, the reduction of BDI scores correlated with the increase of S-100b at 2 h ($r = 0.75$, $P = 0.020$).

TABLE 11 CSF and serum levels of S-100b protein (S-100b) in patients and controls (I)

S-100b µg/l	Patient group (n = 22)	Control group (n = 20)	P value
CSF (mean ± S.D.)	3.2 (1.3)	3.2 (0.9)	0.13
Serum (mean ± S.D.)	0.05 (0.10)	0.08 (0.06)	0.13

TABLE 12 Serial S-100b protein (S-100b) in temporal lobe epilepsy (TLE) and extratemporal epilepsy (XTLE) patients (II) and depressed patients treated with electroconvulsive therapy (ECT) (IV)

S-100b µg/l	0 h	1 h	2 h	3 h	6 h	12 h	24 h	48 h	P value
TLE (n = 15)									
mean	0.068			0.076	0.082*	0.077*	0.064		0.05
(± S.D.)	(0.043)			(0.070)	(0.044)	(0.055)	(0.048)		
XTLE (n = 16)									
mean	0.065			0.064	0.062	0.062	0.057		0.40
(± S.D.)	(0.033)			(0.036)	(0.033)	(0.033)	(0.025)		
ECT (n = 10)									
median	0.16	0.18	0.25		0.14		0.15	0.11	0.55
(quartiles)	0.02-0.41	0.02-0.52	0.07-0.45		0.03-0.62		0.01-0.42	0.03-0.50	

* Wilcoxon matched pairs; 0 h < 6 h, $P = 0.05$; 0 h < 12 h, $P = 0.01$

3. CSF T-tau, P-tau and P-tau/T-tau ratio

There were no statistical differences in T-tau or P-tau levels between different etiologic groups and controls, but P-tau/T-tau ratio differed between the groups and controls (Table 13). The number of seizures, SE or the time interval between the seizures and CSF sample did not correlate with tau concentrations (Spearman's correlation). As the reference values established in our laboratory were for patients over 60 years, we obtained a reference group of younger controls. However, we found no differences in the mean levels of CSF T-tau or P-tau between age-matched controls and patients with epilepsy group 3.

There was statistical difference in the frequency of abnormal T-tau levels between the groups studied (p = 0.002, Pearson Chi-square). None of the patients with epilepsy of unknown origin (group 3) or the controls had abnormal levels of T-tau. Three patients with a single seizure also had normal levels of T-tau (Table 13). However, a total of eleven patients (20%) in other groups had abnormal T-tau levels. The characteristics of these patients are shown in Table 14. The controls differed from groups with symptomatic seizures (groups 1, 2 and 4; p = 0.03-0.003, Fisher's exact test) but not from those with epileptic seizures with unknown etiology (group 3, NS). When groups 2 (SYMPT) and 3 (EPI), i.e. epilepsy without acute illness, were compared, there was also statistical difference in the number of abnormal T-tau (p = 0.04, Fisher's exact test). P-tau was abnormal in four patients and in two controls. Elevations were small except in one patient who was diagnosed with AD.

TABLE 13 The median values of T-tau, P-tau and P-tau/T-tau ratio in different patient groups and controls

	T-tau pg/mL (quartiles)	P-tau pg/mL (quartiles)	P-tau / T-tau ratio (quartiles)
All patients n = 54	163.1 (124.4-328.1)	39.6 (30.2-51.8)	0.23 (0.18-0.25)
Group 1 (ALCO) n = 15	163.1 (129.3-333.3)	36.6 (26.8-53.9)	0.217 (0.164-0.243)
Group 2 (SYMPT) n = 16	259.1 (115.5-518.2)	47.6 (29.1-64.2)	0.202 (0.135-0.242)
Group 3 (EPI) n = 13	133.4 (114.9-159.2)	37.6 (31.1-41.0)	0.266 (0.244-0.327)
Group 4 (ACU) n = 7	251.9 (127.3-388.7)	38.7 (34.2-50.6)	0.191 (0.093-0.232)
Controls n = 31	143.5 (108.1-214.9)	38.1 (31.3-51.6)	0.256 (0.238-0.314)
P - value ANOVA	0.09	0.6	0.003

Group 1 (ALCO), alcohol withdrawal
Group 2 (SYMPT), remote symptomatic epilepsy
Group 3 (EPI), cryptogenic focal, or idiopathic epilepsy
Group 4 (ACU), acute systemic or CNS illness

TABLE 14 Characteristics of the patients with elevated T-tau levels

Patients, age	Diagnosis, etiology of epileptic seizures	CSF T-tau pg/mL	CSF P-tau pg/mL	CSF P-tau/T-tau ratio
Group 1 (ALCO)				
No 1, 44 yrs	Alcoholic encephalopathy	692.0	36.6	0.053
No 2, 69 yrs	Wernicke's encephalopathy	512.3	74.8	0.146
No 3, 41 yrs	Alcohol withdrawal	403.4	69.2	0.172
Group 2 (SYMPT)				
No 4, 88 yrs	Alzheimer's disease	809.0	105.2	0.130
No 5, 62 yrs	Post-traumatic epilepsy	443.3	47.1	0.106
No 6, 86 yrs	Post-stroke epilepsy	1687.9	59.4	0.035
No 7, 32 yrs	Post-encephalitic epilepsy	593.1	83.4	0.141
No 8, 66 yrs	Large inoperable pituitary adenoma	706.2	80.6	0.114
Group 4 (ACU)				
No 9, 16 yrs	Viral encephalitis	388.7	34.2	0.088
No 10, 74 yrs	Viral encephalitis	546.6	50.6	0.093
No 11, 17 yrs	Necrotizing encephalomyelitis	350.2	ND	ND

Group 1 (ALCO), alcohol withdrawal
Group 2 (SYMPT), remote symptomatic epilepsy
Group 4 (ACU), acute systemic or CNS illness

DISCUSSION

The aim of this thesis was to examine possible neuronal injury associated with epileptic seizures. We used established biomarkers NSE, S-100b and tau as indicators of brain damage. The changes in these biomarkers were observed shortly after epileptic seizures in different patient groups and control subjects. We found that single uncomplicated brief seizures did not increase the levels of biomarkers. The effect of seizures in refractory TLE with repeated seizures was different and clear elevations of NSE and S-100b were observed. However, the levels of tau did not increase after idiopathic or cryptogenic epilepsy, and abnormal levels were only found in patients with either acute or remote symptomatic seizures. Although epileptic seizures in ECT did not increase biomarkers, transient elevations of S-100b could indicate glial activation.

This study has some limitations that should be acknowledged. Patient populations in the various groups are small as is usual with clinical settings, especially with studies using CSF measurements. Etiologies of epilepsies and epileptic seizures vary, thus patient groups comprise heterogeneous populations. This could be a confounding factor, even if only adult patients are included. Timing of the samples seems to be critical in analyzing possible transient changes after events that are not long-lasting or ongoing. Serial serum measurements detect transient changes more reliably but LP cannot be repeated straightforwardly. A long-term follow-up of the patients was not a purpose of this study. Methodological issues are discussed further.

1. Methodological considerations

In studies I and III, the control groups comprised patients with acute neurological symptoms (dizziness, headache). These groups may well differ from the normal population even if there was no demonstrable pathology in further examinations. In the control group in study I, the mean CSF NSE values were actually higher (13.0 ng/mL) than in the study group and some individuals had higher levels than upper normal limit. However, no explanation could be given to these high levels; thus the interpretation of only a modest elevation of NSE remains open.

The laboratory has established reference values for tau for older patients (> 60 years) (Herukka et al. 2005), and it is known that CSF tau increases with age (Bürger née Buch et al. 1999; Sjögren et al. 2001). Therefore, we obtained control samples from younger (< 60 years) patients (III). Patient group 3 (EPI) was age-matched with controls; their levels of T-tau or P-tau were not increased compared to the controls. Furthermore, none of the controls had abnormal T-tau or P-tau values.

The patients in video-EEG (II) had refractory epilepsy with usually frequent seizures. Thus it was not possible to determine the effects of single seizure on biomarkers in most cases. Many patients experienced recurrent seizures during the sampling period; therefore, we calculated seizure burden by total duration of seizures recorded during the 24-hour sampling period. However, the number or duration of seizures did not explain the increased levels of biomarkers. Further, we compared individual changes of markers with the baseline levels obtained on admittance to video-EEG.

In the ECT study (IV) the patient population was small. The patient group was also heterogeneous and the samples were obtained after different ECT sessions in the course of the treatment. In this study we assessed possible effects of one session of ECT on serum levels of markers. Some patients had high S-100b levels before ECT but no explanation for that could be found. Possibly, depression itself can be a reason for these high levels (Arts et al. 2006). Propofol, which was used in half of our patients, can shorten seizure duration during ECT (Gábor et al., 2007). However, the changes in biomarkers did not correlate with the duration of seizures or energy used.

The assays used to determine NSE and S-100b concentrations in studies I and IV differed from the one used in study III. Because of the different determination methods the absolute levels are not comparable between the two assays.

2. NSE levels in epileptic and depressive patients

A single uncomplicated tonic-clonic seizure did not induce elevation of the mean CSF or serum NSE obtained in 24 hours after the seizure in patients with newly onset seizures. Two of the three patients who did have increased values had complicated seizures. CSF NSE has been markedly elevated after prolonged seizures in humans and in animal models. Also after single brief seizures CSF NSE increased at 10 to 30 minutes and again at 4 h returning to control levels at 24 h in rat (Oses 2004). Only a few patients have had increased CSF levels after brief seizures measured a few days after the seizure (Jacobi 1988) or immediately after methohexital infusion (Rabinowicz et al. 1994). These patients had a history of epilepsy and in Rabinowicz's study (1994) the patients

were undergoing epilepsy surgery, whereas we evaluated patients who were non-medicated and had the first (or second) epileptic seizure in their life. Single serum measurement of NSE was not increased in our patients with no history of epilepsy and CSF and serum levels did not correlate. NSE is thought to be released into the CSF compartment by an increase in permeability of the BBB. The rate of release and clearance of NSE from CSF and serum is largely unknown. As our samples were taken within 24 h after seizure (mean 15.1 h), possible elevations after that time went undetected especially in serum samples.

Serial measurements of serum NSE showed significant changes in refractory epilepsy patients with TLE. NSE levels started to increase at 3 h after seizure and the time to reach maximum was 10.2 hours in the TLE group. The postictal levels in XTLE showed an initial trend towards an increase at 3 hours, but it was not statistically significant. Other studies have found increases after brief seizure in some patients. In a study similar to ours, NSE levels were increased in only a few of the patients, and the mean levels of NSE were not significantly increased (Tumani et al. 1999). The authors did not compare the results between the TLE and XTLE groups, nor did they measure baseline levels but compared the postictal NSE levels with those of control subjects. Furthermore, the levels in the complex partial seizure (CPS) and SGTCS groups did not differ, but the NSE values peaked later in the SGTCS group (3 h vs 1 h) (Tumani et al. 1999). In one study, which did not provide information on the localization of the seizure focus, elevated serum NSE levels were observed in altogether 34 % of patients (both CPS and SGTCS) (Willert et al. 2004). Another study found increased serum levels of NSE both after single CPS (3 of 9) and single SGTCS (4 of 4) (Rabinowicz et al. 1996). Only five of our patients had SGTCS and their NSE levels did not explain the changes observed.

Most of the patients had CPS as index seizure, indicating that CPS can produce marked elevations of this marker. There is evidence that high lifetime seizure number and longer duration of epilepsy contributes to more severe neuronal damage (Van Paesschen et al. 1997; Salmenperä et al. 2001; Kälviäinen and Salmenperä 2002; Mathern et al. 2002). We did not find a correlation with the duration of epilepsy and the increase of NSE in TLE.

Serial measurements of serum NSE after ECT remained unchanged, suggesting that ECT does not induce neuronal injury. This is in accordance with previous studies, which have shown no systematic ECT-induced serum NSE changes (Greffe et al. 1996; Berrouschot et al. 1997; Agelink et al. 2001). Recent animal studies suggest that ECS, the experimental analogue of ECT, causes both structural and functional changes within the hippocampal formation (Lamont et al. 2005). Repeated ECS induces sprouting of mossy fibers (Gombos et al. 1999) along with neurogenesis (Scott et al. 2000) and increased expression of NSE (Rasmussen et al. 1994). Clinical relevance of these findings is not clear. There is a report of a post-mortem brain study on a patient with over 1250 ECTs. No gross or histological signs of brain damage or development of epilepsy was observed (Lippman et al. 1985).

It is still controversial whether epileptic seizures, especially brief recurrent seizures, damage the brain. There is no question that some seizures affect the structure of the brain and that in some patients epilepsy becomes progressive, more commonly in TLE. Whether the damage seen in the hippocampus in epilepsy patients is the cause or the consequence of TLE is a fundamental question. There is evidence that the damage in the medial temporal lobe may be either of these (Kälviäinen and Salmenperä 2002; Mathern

et al. 2002). The levels of NSE did not increase in our patients with single uncomplicated tonic-clonic newly onset seizures but were clearly increased in refractory TLE patients with repeated seizures. Mesial temporal structures are more prone to damage than are other areas of the brain (Holmes 2002). Thus, the risk of seizure-related neuronal injury may be different in TLE from that in XTLE or in newly onset seizures. The increase in NSE after seizure was transient and less than that seen in severe brain damaging conditions, such as TBI and stroke. The increase in NSE in epileptic patients might indicate a more subtle and transient injury. Exposure to repetitive mild head injuries is considered to have cumulative effects that may result in long-term cognitive dysfunction (Weber 2007; Shuttleworth-Rdwards and Radloff 2008). Similarly, in patients with refractory epilepsy the cumulative effect of even transient brain injury associated with seizures might become clinically significant in the course of years.

3. S-100b levels in epileptic and depressive patients

Single uncomplicated tonic-clonic seizure did not induce increase in the mean CSF or serum S100b after the seizure in patients with newly onset seizures. Few studies have measured serum S-100b after single seizures with similar findings (Büttner et al., 1999; Leutmezer et al., 2002). Previously, no measurements of CSF S-100b have been made after epileptic seizures.

The change in the serum levels of S-100b was less pronounced and of shorter duration than the change in NSE in refractory TLE patients with repeated seizures. Peak levels of S-100b in TLE-related seizures seemed to occur at 6 hours postictally. In contrast to our

findings, the only previous study of S-100b in TLE patients in video-EEG did not find changes in S-100b (Leutmezer et al. 2002). As the number of patients was small (n=10), different results might be explained by a random sampling effect and differences in the methods used for biochemical analyses. In our patients, S-100b levels were more increased at 3 h after SGTCS than after CPS (0.135 µg/L vs. 0.059 µg/L). The number of SGTCS patients was small, so no conclusions can be made based on this finding.

The median change in S-100b was not statistically significant in patients after ECT, which supports previous evidence of the safety of ECT. There was, however, a transient increase in the S-100b levels in four out of the ten patients studied. Only one previous study using S-100b measurements has found a rise in S-100b levels at 1 and 3 hours after ECT (Arts et al. 2006). Previous studies used different time points for S-100b measurements (Agelink et al. 2001, Zachrisson et al. 2000; Arts et al. 2006), so possible transient changes may not have been detected in these studies compared with our more frequent sampling. We did not see substantial changes in S-100b that would indicate brain damage but rather more subtle and transient increases possibly indicating glial activation. Agelink et al. (2001) reported that patients with higher post-ECT values of NSE and S-100b showed the best cognitive test performance. These findings have led to a suggestion that ECT-induced release of S-100b may promote neural plasticity and may play a part in mediating the antidepressant and cognitive effects of ECT (Agelink et al. 2001; Arts et al. 2006). Supporting this, in our study, high S-100b levels at 2 h and 6 h correlated with the treatment response and also the increase at 2 h with a reduction of BDI scores. The median changes in S-100b concentrations within the ECT group, according to our present results, seem to be not as important as changes associated with ECT treatment responses. Newer findings report that S-100b might be used as a

biomarker for mood disorders and their treatment (Schroeter et al 2008). Our results support this hypothesis, as the most important finding of our study was the relation between S-100b and a positive treatment effect.

Neuronal function is tightly modulated and controlled by a number of non-neuronal cells. Many of these are glial cells, which participate actively in synapse development and in regulation of BBB function, and play prominent roles in neurodegenerative processes (Schwarcz 2008). S-100b has been used as a marker of brain damage and pathological concentrations have been found in many CNS disorders (Sen and Belli 2007). Recently, S-100b has gained increasing interest as a marker of glial functions. Newer studies suggest that it could be a marker of beneficial reactions and be even neuroprotective. In pathological conditions it would be useful to determine both S-100b and NSE concentrations. With unaltered NSE levels, increased S-100b levels would rather indicate glial activation and, depending on S-100b concentration, the effects could be either trophic or toxic.

4. T-tau, P-tau and P-tau/T-tau ratio after epileptic seizures

CSF tau levels after seizures were within the normal range in patients with idiopathic or probably symptomatic (cryptogenic) epilepsy, and abnormal levels were only found in patients with either acute or remote symptomatic seizures. It seems that epileptic seizure itself does not increase CSF tau if there is no other profound pathology in CNS. There is only one case report based on a single epilepsy patient who was found to have a transient elevation of CSF tau measured four days after the seizure compared with the baseline

value (Matsui et al. 2007). This patient had a history of idiopathic normal-pressure hydrocephalus with dementia. It is possible that the increase in CSF tau in this patient was the result of both GTCS and underlying pathology. To our knowledge seizure-related changes of CSF tau have not otherwise been studied in humans. The reduction of endogenous tau levels protected mice against excitotoxicity in an AD mouse model (Roberson et al. 2007), indicating that tau may mediate excitotoxicity. Therefore high CSF tau levels after seizure could mark a poorer prognosis. In our patients, the number of seizures or SE did not correlate with the levels of tau.

It has been suggested that elevated CSF T-tau levels are useful in differentiating AD from normal ageing and depression (Blennow et al. 2006). However, an overlap exists with other neurodegenerative disorders or conditions with brain parenchymal damage (Blennow et al. 1995; Kapaki et al. 2007). P-tau is a more specific marker for AD reflecting the phosphorylation state of tau. Consistent with this, P-tau levels were not increased in stroke or in CJD disease (Hesse 2001; Riemenschneider 2003). One of our patients was diagnosed with AD and had clear T-tau and P-tau abnormalities. Otherwise, three patients had slightly abnormal P-tau levels but no symptoms suggesting AD pathology. All our patients with abnormal T-tau levels had symptomatic etiologies for epileptic seizures. These included alcoholic encephalopathy, CNS infections and various remote symptomatic causes. Previously, elevated CSF T-tau levels have been noted in patients with alcohol-related cognitive disorders and in chronic alcoholics with thiamine deficiency. Degeneration of nucleus basalis may develop through formation of neurofibrillary tangles (Cullen and Halliday 1995). Increased levels of tau have also been reported in patients with cerebral infections. In HIV-infected patients who had cerebral infections and necrosis, elevated tau levels were found in 35 % (Green et al.

2000). Patients with viral encephalitis and bacterial meningitis with meningoencephalopathic complications had elevated tau levels (Süssmuth et al. 2001). This suggests that tau may be a marker for parenchymal involvement in cerebral infections.

In Kapaki's study (2007) sensitivity and specificity of P-tau/T-tau ratio were better in the discrimination of AD versus controls than P-tau alone. The best cutoff level for this ratio was ≥0.169. Most of our patients with abnormal T-tau levels had quite low P-tau and also low P-tau/T-tau ratio, indicating rather non-specific neurodegenerative disorder or axonal damage. Thus, P-tau/T-tau ratio does not seem to be a useful marker in discriminating pathological conditions in this patient population.

The rate of release of tau in acute brain-damaging conditions is largely unknown. CSF tau showed an increase at 2-3 days after acute stroke (Hesse et al. 2000; 2001) but in TBI and brain injury after aortic surgery the increase in tau levels started earlier, during the first day (Zemlan et al. 1999; Franz et al. 2003; Shiiya et al. 2004). The time interval between LP and seizure varied (mean 14.4 h) in our patients but no correlation between tau levels and the time interval was observed.

CSF T-tau seems to be a non-specific marker of brain damage, especially in neurodegenerative disorders and axonal damage. P-tau is a more specific marker for neurofibrillary pathology. Abnormal CSF T-tau levels may be found in patients with seizures. However, acute seizure without profound pathology in CNS itself does not seem to lead to elevation of CSF T-tau. Acute symptomatic seizures due to alcohol withdrawal are among the most common etiologies in emergency departments,

(Forsgren et al. 1996). CSF tau measurement could provide a tool in assessing these patients for further evaluation. The presence of increased CSF tau in a patient with epileptic seizure increases the probability of symptomatic cause. Further etiologic investigations are warranted in such patients. Elevated levels of tau in epileptic patients might implicate more frequent follow-up in these patients. Abnormal tau levels can predict further development of dementia disorder.

5. Future implications

Epilepsy and epileptic seizures affect a substantial number of people world wide. Although 60-70 % of patients with epilepsy become seizure free, a considerable proportion of epilepsy patients suffer from refractory epilepsy and have recurrent seizures. Some of them have progressive features such as increasing seizure frequency and cognitive decline. There is no question that some seizures affect the structure of the brain, induce neuronal damage and chronic changes. Our results support earlier observations. The main finding of our study was that single uncomplicated seizures do not induce elevations of biomarkers but repeated seizures, especially in TLE, do. It would be beneficial to be able to identify those patients who are at risk for damage and progression. This information might have a reflection on treatment, i.e. proceeding to surgery or vagus nerve stimulation earlier in these patients. NSE and S-100b might prove useful in identification of more severe forms of epilepsies. A proportion of refractory epilepsy patients admitted to video-EEG monitoring would be further selected for epilepsy surgery. Prospective studies reporting seizure type, seizure number and the levels of biomarkers in patients undergoing epilepsy surgery would

permit precise correlations between clinical seizure types, biomarkers and neuronal death mechanisms. This might offer novel targets for therapies aimed at reducing seizure-induced damage and epileptogenesis.

ECT is considered a safe and effective treatment. However, not all patients with treatment resistant depression benefit from this treatment. A marker that would single out those patients who would gain a positive treatment effect would be of great interest. A prospective study of S-100b with a more homogeneous patient group admitted to ECT is needed.

Experimental animal studies have shown that complex reorganization occurs during epileptogenesis, such as neurogenesis, glial proliferation, axonal plasticity, inflammation, and neuronal loss. There is also evidence of continuing reorganization due to recurrent seizures (Pitkänen and Sutula 2002). Seizure-induced plasticity in neural circuits is not always harmful and can be even protective (Sutula 2004). The search for molecular mechanisms underlying seizure-related neuronal injury and progressive brain-function decline in patients with seizures will reveal opportunities to prevent both structural damage and progression in epileptic seizures (Pitkänen and Sutula 2002). Reliable and well-validated biomarkers for epilepsy offer the potential to provide a window into the disease mechanism and its progression and to potentially generate therapeutic targets for treatment. In the future, searching for more specific biomarkers, which might be found among indicators of neurogenesis, apoptosis or inflammation and cytokines, is needed.

SUMMARY AND CONCLUSIONS

1. Single tonic-clonic seizures are not associated with elevations of NSE or S-100b in patients with no history of epilepsy, but localization-related refractory epilepsy induces increase in these biomarkers. This finding indicates that uncomplicated brief newly onset seizures may not induce neuronal or glial damage. It is possible that the effect of seizures in refractory epilepsy with repeated seizures differs from that in newly onset single seizures.

2. The patients with TLE showed elevation of both markers of brain damage, NSE and S-100b, after epileptic seizure, whereas in XTLE the changes were shorter in duration and of no significance. The levels of NSE peaked approximately at 10 hours. It is possible that in refractory TLE the sensitivity to damage is different than in other localization-related epilepsies, and the subtle damage induced by recurrent brief seizures over the years contributes to the progression of the disorder.

3. Epileptic seizures with no recognizable etiologic factor do not induce elevation of T-tau or P-tau protein. However, some patients with epileptic seizures with different underlying etiologies have increased levels of T-tau, suggesting that these patients have a neurodegenerative disorder or axonal damage due to various neurological conditions.

4. ECT does not seem to induce neuronal injury determined by measurements of NSE and S-100b. Clear transient elevation of S-100b after ECT in four out of ten patients suggests glial activation, and high S-100b levels correlated with treatment response. This activation could be neurotrophic in nature and the antidepressant effect of ECT may be mediated by glial activation.

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Normal CSF neuron-specific enolase and S-100 protein levels in patients with recent non-complicated tonic–clonic seizures

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Abstract

Purpose: Increased concentrations of the nervous-system-specific proteins neuron-specific enolase (NSE) and S-100 protein (S-100) have been measured with lesions in the CNS. Elevated levels of serum NSE (s-NSE) have been found in status epilepticus, but also after single epileptic seizures. Because larger studies addressing cerebrospinal fluid (CSF) levels of NSE or S-100 have not been performed, we measured CSF NSE and S-100 after tonic–clonic seizures to search for evidence of neuronal and glial damage. *Methods:* 22 consecutive patients with single, previously undiagnosed and untreated tonic–clonic seizures were studied. Serum and CSF samples were collected within 24 h after seizure. 18 serum and CSF samples were measured from a control group. *Results:* The mean CSF NSE was 8.9 ng/ml (range 0–28 ng/ml) and s-NSE 8.2 ng/ml (range 5–15 ng/ml) in the patient group. The mean concentrations in the control group were 13.1 ng/ml (range 3–24 ng/ml) and 8.0 ng/ml (range 5–12 ng/ml) respectively. The mean CSF S-100 was 3.17 μg/l (range 1.45–7.02 μg/l) and serum S-100 0.05 μg/l (range 0–0.32 μg/l), and in controls 3.19 μg/l (range 1.52–5.13 μg/l) and 0.08 μg/l (range 0–0.28 μg/l). *Conclusion:* There were no significant differences between the mean concentrations of NSE or S-100 in CSF and serum between the epileptic group and controls. These results do not confirm the previous observation of elevated NSE-levels after tonic–clonic seizures, which argues against neuronal or glial damage after uncomplicated tonic–clonic seizures in unmedicated patients. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Neuron-specific enolase; S-100 protein; Cerebrospinal fluid; Epilepsy

1. Introduction

Acute brain injury caused by status epilepticus (SE) has been well documented both in humans and experimental models, but whether single generalized tonic–clonic seizures can damage the brain is controversial [1]. Neuron-specific enolase (NSE) and S-100 protein (S-100) are regarded as nervous-system-specific proteins. NSE, γ-subunit of enolase, is predominantly present in neurons,

neuroendocrine cells and neuroendocrine tumors. S-100 is a mixture of dimeric proteins consisting of two subunits α and β. S-100b (ββ-S-100) is present in high concentrations in glial cells and Schwann cells.

Cerebrospinal fluid neuron-specific enolase (CSF NSE) has been the best marker of neuronal damage in neurologic insults such as stroke and anoxia [2], but there are only a few studies addressing CSF NSE concentrations in epilepsy. CSF NSE was increased after methohexital-induced electrographic seizures in patients undergoing epilepsy surgery [3]. Elevated CSF NSE levels have been reported in children with afebrile seizures [4] and in 4 out of 9 adult patients who suffered epileptic seizures within 5 days of

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lumbar puncture [5]. CSF obtained within 24 h of status epilepticus showed increased concentrations of NSE in 9 out of 11 patients [6]. Serum NSE (s-NSE) was increased after tonic–clonic SE [7], complex partial SE [8] and nonconvulsive SE [9]. When determining s-NSE levels in the major subtypes of SE, DeGiorgio et al. found these levels to be highest in complex partial and subclinical generalized convulsive SE [10]. Elevated levels of s-NSE have been observed after single seizures during inpatient video/EEG monitoring, suggesting that also single seizures could cause at least transient neuronal injury [1].

Increased levels of S-100 in CSF or serum have been measured after focal and global ischemic or hemorrhagic brain damage and head trauma indicating damage in glial cells [11–14]. In patients with hypoxic brain damage after cardiac arrest S-100 levels have correlated with the degree of coma and the time of anoxia [13]. There has also been a correlation between higher peak S-100 levels and worse clinical outcome both at discharge and in long-term follow-up [14]. S-100 concentrations in serum showed peak levels within 1–3 h after an epileptic seizure [15]. Steinhoff et al. measured cisternal CSF S-100 and NSE levels obtained during implantation of foramen ovale electrodes in eight patients with temporal lobe epilepsy. These levels in ipsilateral site of seizure onset were significantly higher than in controls [16].

We studied CSF and serum levels of NSE and S-100 after recent unprovoked single tonic–clonic seizures. At least in serum, these measurements are simple, fast and easy to carry out, and they might be helpful in evaluating the occurrence and degree of neuronal and glial damage after non-complicated seizures and in designing treatment in acute epileptic seizures [1], but such studies have not been undertaken to our knowledge.

2. Patients and methods

A total of 22 consecutive patients with single, previously undiagnosed and untreated tonic–clonic or partial secondarily generalized seizures [17] were studied. The mean age in the epileptic group was 39 years (range 15–60) and in the control group 40 years (16–56 years). The serum and CSF samples of NSE and S-100 were taken within 24 h after the seizure. We excluded patients with seizures associated with electrolyte disturbances, metabolic causes, acute brain disease and trauma. Hemolyzed samples were not included for NSE analysis. All patients were fully informed of the risks and potential benefits of the CSF examination, and informed consent was obtained from each subject. The study protocol was approved by the Ethics Committee of Tampere University Hospital. The control samples were obtained from 18 adult patients on whom lumbar puncture (LP) was performed to exclude neurological disease. These patients had normal results in neurological examination and normal radiology (CT or

MRI imaging of the brain in 10 out of 18) and laboratory findings. Lumbar CSF was obtained between 9 a.m. and 2 p.m. The first 2 ml of CSF was used for routine examination and a further 200 μl for the present study. Blood was collected within 30 min of lumbar puncture in a Vacutainer EDTA vacuum tube and centrifuged at 3000 rpm for 10 min. The serum and CSF samples were stored at −70°C prior to analysis.

NSE assays were performed using an enzyme immunoassay technique (Cobas Core® NSE EIA, Hoffmann-La Roche, Switzerland). S-100b concentrations were measured with immunoluminometric assay for the quantification of protein (LIA-mat® Sangtec®100, Sangtec Medical, Sweden). The assays were performed according to manufacturers' instructions. The sensitivity of the S-100 assay was <0.02 μg/l.

2.1. Statistical methods

The mean and standard deviations were calculated for continuous variables. Statistical significance of differences between two groups was tested by independent two-tailed *t*-test. Associations between continuous variables were assessed with Pearson's correlation coefficient. All analyses were performed using a microcomputer and the Statistica/WIN package (version 5.1; Statsoft Inc., Tulsa, OK). A *P* value of less than 0.05 was considered statistically significant.

3. Results

The mean age in the epileptic group was 39 years (range 15–60) and in the control group 40 years (16–56 years). 12 patients had single seizures. Six patients had seizures due to alcohol withdrawal. All patients had had tomoclonic or partial secondarily generalized seizures and six patients had another seizure in emergency room. The seizures lasted from 1 to 15 min according to inpatient records. After the first epileptic seizure patients usually underwent EEG and CT or MRI examinations. There were two cerebral tumors (meningeoma and glioblastoma multiforme) in the epileptic group. The other patients had normal CT/MRI findings. The serum and CSF samples of NSE and S-100 were taken within 24 h after the seizure (mean 15.1 h, range 2.5–23 h). There were no significant differences in the mean values of CSF and serum NSE or CSF and serum S-100 between epileptic and control groups (Table 1). These values for individual patients and controls are given in Fig. 1. Three patients had CSF NSE levels above 12 ng/ml. One patient whose level was 28 ng/ml had an additional prolonged seizure in emergency room (the first seizure of some min at home and the second lasting 15 min). Another patient (CSF NSE level 23 ng/ml) had also two seizures within 24 h regaining consciousness between them but having difficulties in

Table 1
CSF and serum neuron-specific enolase (NSE) and S-100 levels in patients with recent non-complicated tonic–clonic seizures

	Controls (n=20)	Patients (n=22)	P values
CSF[a]-NSE (ng/ml)	13.1	8.9	0.60
(S.D.)[a]	(6.1)	(6.9)	
s-NSE (ng/ml)	8.0	8.2	0.21
(S.D.)	(2.1)	(2.8)	
CSF-S-100 (µg/l)	3.19	3.17	0.13
(S.D.)	(0.92)	(1.33)	
s-S-100 (µg/l)	0.08	0.05	0.13
(S.D.)	(0.06)	(0.10)	

[a] CSF, cerebrospinal fluid; S.D., standard deviation.

speaking for some time after the seizures. The third patient who had the level 19 ng/ml had had only one short uncomplicated seizure. The two patients who were found to have tumors (meningeoma and glioblastoma multiforme) also had normal values (in CSF, NSE 3 and 9 ng/ml, and S-100 3.16 and 2.91 µg/l respectively).

There was also no correlation between serum and CSF levels of NSE ($r=0.13$, $P=0.40$) and serum and CSF levels of S-100 ($r=0.05$, $P=0.73$)

4. Discussion

To our knowledge, this study is the first to report that CSF and serum NSE levels are not elevated after uncomplicated seizures in newly diagnosed patients. The results are in contrast to those of Rabinowicz et al. [1] who reported elevation of s-NSE; the mean baseline s-NSE was 7.6 ng/ml and it increased to 16.5 ng/ml in four patients after secondarily generalized tonic–clonic seizures. In our study the mean s-NSF level in the patient group was 8.2 ng/ml and the mean CSF-NSE level was 8.9 ng/ml; these values are comparable to the baseline value in Rabinowicz's study [3]. The main reason for this discrepancy may be difference in patient population. We evaluated patients who were nonmedicated and had their first (or second) epileptic seizure in their life whereas the patients in Rabinowicz's study were evaluated for epilepsy surgery. It is possible that the effect of seizures in chronic epilepsy may differ from that in newly onset single seizures or epilepsy. Patients with chronic refractory epilepsy show a decrease in hippocampal volume possibly as a consequence of seizures [18]. Although it has been shown that a single episode of status epilepticus may lead to hippocampal damage, it remains open whether a short, uncomplicated seizure can lead to neuronal damage. A high proportion of patients with convulsive and non-convulsive SE had an elevation of s-NSF suggesting that the duration of the clinical or electrical seizure activity may be of crucial importance in determining whether neuronal damage occurs [7,8].

DeGiorgio et al. [10] found highest s-NSE levels in
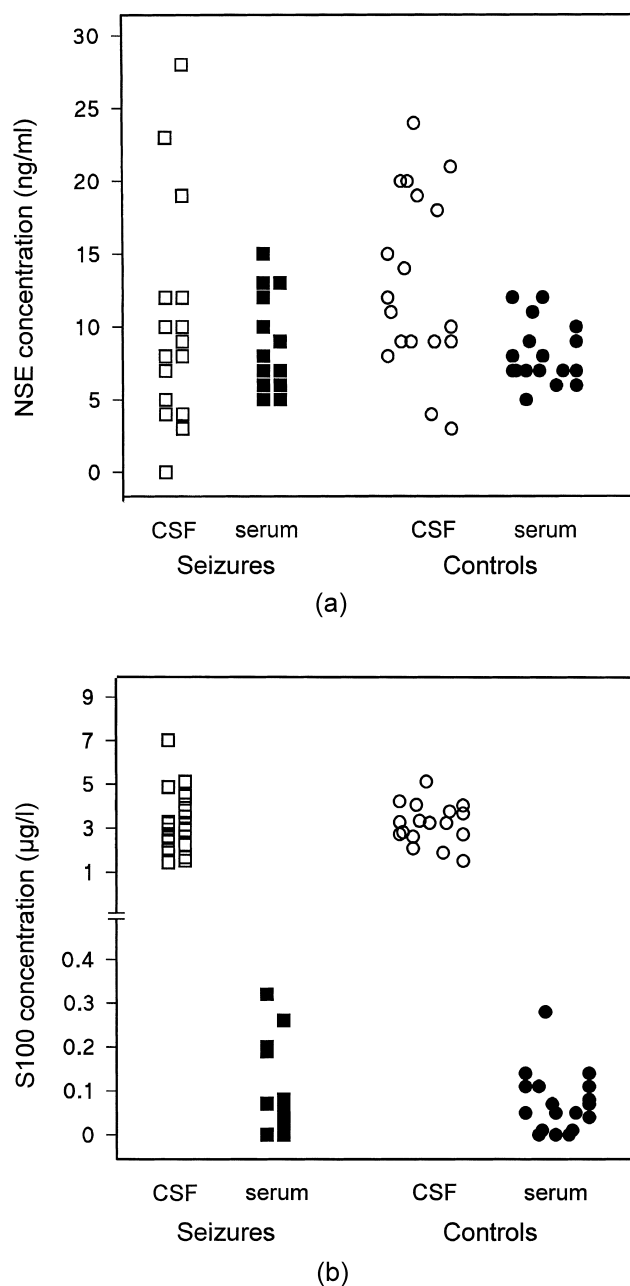
Fig. 1. (a) NSE concentrations in patients with seizures and in controls (b) S-100 concentrations in patients with seizures and in controls.

complex partial and subclinical SE, attributing these findings to longer duration of SE and acute neurological insults in these subgroups. Our patients had only tonic–clonic or partial secondarily generalized seizures. One patient whose CSF NSE level was 28 ng/ml had an additional prolonged seizure lasting 15 min in emergency room. Another patient (CSF NSE level 23) had also two seizures within 24 h regaining consciousness between them but having difficulties in speaking for some time after the seizures. These factors might explain the high levels of CSF NSE of these two patients, which would seem to indicate that duration of seizures is an important factor.

Theoretically, NSE is an ideal indicator of neuronal damage, since it is present only in low concentrations outside the nervous system. CSF NSE has been the best indicator of neuronal damage in such disorders as anoxic brain damage after cardiac arrest, stroke and some specific cases of coma [2]. In these conditions s-NSE levels have correlated with CSF values rather well. It has been suggested that seizure activity in the brain causes a release of NSE into the CSF compartment. S-NSE is thought to be released into the CSF compartment by an increase in permeability of the blood–brain barrier. The rate of release and clearance of NSE from CSF and serum are largely unknown. In three patients CSF NSE levels rose three- to fourfold from baseline within 60 min after methohexital activation but s-NSE was unchanged [3]. In Rabinowicz's study s-NSE levels attained peak levels 24–48 h after a seizure [1]. In Thumani's study s-NSE levels were highest at 1 h in the group of patients with complex partial seizures and at 3 h in the group with secondarily generalized tonic–clonic seizure [19]. DeGiorgio et al. [10] reported also that s-NSE peaked on average 41 h after the onset of SE and 41% of subjects peaked within 24 h and 33% within 24 to 48 h. As our samples were taken within 24 h after seizure (mean 15.1 h), possible elevations after that time went undetected.

The definitions of normal upper limits are quite variable depending on the control group chosen. In our study the control group comprised patients with acute neurological symptoms (dizziness, headache). This group may well differ from the normal population even if there was no demonstrable pathology in further examinations. CT/MRI imagings were made in 10 patients out of 18 and the results were all normal. In the control group the mean CSF values were actually higher (13.0 ng/ml) than in the study group. The highest CSF NSE value in the control group was 24 ng/ml. Slightly raised CSF NSE levels have been reported in conditions like migraine that are not traditionally associated with neuronal damage [5], so the interpretation of modest elevation of NSE remains open.

S-100 levels have been studied mostly in patients with acute brain infarction, hypoxic brain damage after cardiac arrest, and head trauma. In eight patients with brain infarction whose S-100 levels were measured daily for 9 days, peak S-100 plasma levels were measured 2.5 ± 1.3 days after infarction [14]. On the other hand there were peak levels of S-100 in serum within 1–3 h after complicated partial and secondarily generalized epileptic seizure and a gradual decrease thereafter [15]. These differences may be due to different mechanisms in epileptic seizure and brain damage after ischemic incident. In our study measurements were made within 24 h after the seizure and there were no elevated concentrations in the patient group.

In conclusion, our study reported normal CSF NSE and S-100 levels after noncomplicated single seizures. Larger series with different epileptic groups are needed to determine the incidence of increase in concentrations of these indicators of neuronal and glial damage. No clear correlation between serum and CSF levels of NSE or S-100 emerged with these normal results. Increased concentrations of NSE and S-100 would be needed to ascertain whether there is a correlation between serum and CSF levels in conditions such as status epilepticus.

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Elevated serum neuron-specific enolase in patients with temporal lobe epilepsy: A video—EEG study

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Summary Established markers of brain damage, neuron-specific enolase (NSE) and S-100b protein (S-100), may increase after status epilepticus, but whether a single tonic-clonic or complex partial seizure induces elevation of these markers is not known. Furthermore, it is unclear whether the risk of seizure-related neuronal damage in temporal lobe epilepsy (TLE) differs from that in extratemporal lobe epilepsies (XTLE). The aim of this study was to analyze NSE and S-100 in patients with TLE and XTLE after acute seizures. The levels of NSE and S-100 were measured in serum before (0 h) and at 3, 6, 12, and 24 h after acute seizures in 31 patients during inpatient video—EEG monitoring. The patients were categorized into the TLE and the XTLE group based on video—EEG recordings and MRI findings.

Fifteen patients had TLE and 16 XTLE. Index seizures were mainly complex partial seizures ($n = 21$). In TLE mean $\pm$ S.D. values for NSE levels (μg/L) were 8.36 ± 2.64 (0 h), 11.35 ± 3.84 (3 h), 13.48 ± 4.49 (6 h), 12.95 ± 5.46 (12 h) and 10.33 ± 3.13 (24 h) ($p = 0.006$, ANOVA). In XTLE the changes were not significant ($p = 0.3$). There was less increase in the levels of S-100 in TLE ($p = 0.05$) and no significant change in XTLE ($p = 0.4$).

The levels of markers of neuronal damage were increased in patients with TLE, not only after tonic-clonic but also after complex partial seizures. These data suggest that TLE may be associated with brain damage.
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Introduction

NSE and S-100 are specific markers of brain damage. NSE, the γ-subunit of enolase, is predominantly present in neurons, neuroendocrine cells and neuroendocrine tumors. S-100 is present in high concentrations in glial cells and Schwann cells. Measurement of the serum levels of these proteins can be used to assess the primary damage as well as to predict outcome after hypoxic (Böttiger et al., 2001; Rech et al., 2006) and traumatic brain damage (Vos et al., 2004) and ischemic stroke (Wunderlich et al., 1999).

Both experimental and clinical studies have shown that status epilepticus may induce neuronal damage, especially in the hippocampus (DeGiorgio et al., 1995; Correale et al., 1998; Young, 2006). Whether repeated brief seizures may cause neuronal damage is, however, controversial (Pitkänen et al., 2002; Sutula, 2004). Increased levels of NSE have been reported after single seizures during inpatient video—EEG monitoring (Rabinowicz et al., 1996) and the S-100 concentrations in serum have peaked within 1—3 h after an epileptic seizure (Otto et al., 1998). However, in our previous study neither NSE nor S-100 was elevated in serum or CSF after a single tonic-clonic seizure in patients with a newly diagnosed seizure disorder (Palmio et al., 2001). The studied seizure type has mainly been generalized tonic-clonic seizure; only a few analyses have been done after partial seizures. No comparison between temporal lobe epilepsy (TLE) and extratemporal lobe epilepsies (XTLE) regarding NSE or S-100 has been made. There is clinical and experimental evidence suggesting that TLE may be a seizure-related progressive disorder (Pitkänen and Sutula, 2002), but data on XTLE are lacking.

The aim of this study was to analyze NSE and S-100 levels in patients with TLE and XTLE before and after acute seizures, observed in video—EEG monitoring, so as to determine whether acute seizures induce neuronal damage.

Methods

The study was performed at the Department of Neurology, Tampere University Hospital, Finland. The patients were selected from those who were admitted to 24-h inpatient video—EEG monitoring for electro-clinical characterization of seizures, localization of the seizure focus, or for therapeutic assessment. Seizures and epileptic syndromes were classified according to the ILAE diagnostic criteria (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). We included 31 patients with localization-related epilepsy, 16 women and 15 men, with a mean age of 34 years (range: 16—58 years). Based on the findings in the video—EEG recordings and MRI, 15 of the patients had TLE and 16 had XTLE. Of the XTLE patients 14 had frontal lobe epilepsy and 2 had parietal lobe epilepsy. Clinical data of these two groups of patients are shown in Table 1. Seizure burden was measured by total duration of seizures recorded during the 24 h sampling period. Six of the patients were on antiepileptic drug monotherapy, 24 on polytherapy (the mean number of medications 2.6), and 1 patient had no medication. Three of the patients were also treated with vagus nerve stimulation.

All the patients gave their written informed consent. The study protocol was approved by the Ethics Committee of the Tampere University Hospital.

Blood sampling and biochemical analyses

The baseline (0 h) serum samples were collected when the patients were admitted for video—EEG monitoring, and at 3, 6, 12, and 24 h after index seizure (Table 1). The first clearly identifiable ictal event was selected as index seizure. The serum samples were stored at −70 °C prior to analysis. NSE and S-100 assays were performed using electrochemiluminescense immunoassay techniques (ECLIA) (Roche Diagnostics GmbH). The NSE and S-100 concentrations were measured by the Elecsys® 2010 Immunoassay Analyzer (Roche Diagnostics GmbH). The lower detection limit for NSE was <0.05 μg/L and the lower detection limit for S-100 was <0.005 μg/L.

Table 1   Clinical characteristics of patients in video—EEG

	Temporal lobe epilepsy	Extratemporal epilepsy
No. of patients	15	16
Male/female	8/7	7/9
Age, mean (range)	40.3 (20—58)	28.3 (16—52)
Mean duration of epilepsy, years (range)	24.2 (2—56)	16.2 (1—52)
Mean seizure frequency/month[a] (range)	7.4 (0.5—26)	40.8 (0.5—200)
Patients on mono-/polytherapy	3/12	3/12
MRI findings		
Normal	4	8
HS	9	—
Cortical dysplasia	1	4
Other	1	4
Index seizure type		
SPS	1	4
CPS	11	10
SGTCS	3	2

HS, hippocampal sclerosis; SPS, simple partial seizure; CPS, complex partial seizure; SGTCS, secondary generalized tonic-clonic seizure.
[a] During the last year.

Table 2 The mean values of NSE and S-100 (μg/L $\pm$ S.D.) in temporal lobe (TLE) and extratemporal lobe epilepsy (XTLE) before (0 h) and at 3, 6, 12 and 24 h after index seizure

	0 h	3 h	6 h	12 h	24 h	p-value ANOVA
NSE						
TLE	8.36 $\pm$ 2.64	11.35 $\pm$ 3.84[a]	13.48 $\pm$ 4.49[a]	12.95 $\pm$ 5.46[a]	10.33 $\pm$ 3.13[a]	0.006
XTLE	9.44 $\pm$ 1.52	11.34 $\pm$ 3.52	10.33 $\pm$ 3.41	10.09 $\pm$ 2.81	9.38 $\pm$ 2.67	0.3
S-100						
TLE	0.068 $\pm$ 0.043	0.076 $\pm$ 0.070	0.082 $\pm$ 0.044[b]	0.077 $\pm$ 0.055[b]	0.064 $\pm$ 0.048	0.05
XTLE	0.065 $\pm$ 0.033	0.064 $\pm$ 0.036	0.062 $\pm$ 0.033	0.062 $\pm$ 0.033	0.057 $\pm$ 0.025	0.4

[a] Wilcoxon-matched pairs: 0 h < 3 h, $p = 0.005$; 0 h < 6 h, $p = 0.0009$; 0 h < 12 h, $p = 0.002$; 0 h < 24 h, $p = 0.008$.
[b] Wilcoxon-matched pairs: 0 h < 6 h, $p = 0.05$; 0 h < 12 h, $p = 0.01$.

Statistical analysis

Friedman analysis of variance (ANOVA) with post hoc comparisons (Wilcoxon matched pairs test), Kruskall—Wallis ANOVA and Mann—Whitney U-test were used to compare concentrations of NSE and S-100. Area under curve (AUC) values were estimated using MedCalc (ver. Win 9.1.0.1, MedCalc software, Mariakerke, Belgium) (Matthews et al., 1990). Statistical calculations were carried out using Statistica (ver. Win 5.1D, Statsoft Inc., Tulsa, OK, USA). Findings were considered statistically significant at p values less than 0.05.

Results

In the TLE group the serum levels of both NSE and S-100 showed a statistically significant increase, whereas in the XTLE group the changes were not significant. The mean values for NSE and S-100 in the two groups are shown in Table 2 and the individual values for NSE in Fig. 1. As the increase in the NSE concentration after an index seizure seemed to be single peaked and linear, we made a further effort to analyze NSE kinetics during the different time intervals by calculating the AUC curves for each subject (Matthews et al., 1990). As could be expected, based on the data of single time points (Table 2), the difference in NSE AUCs from the baseline (0 h) to the 24 h end-point was clearly significant (AUC$_{0-24h}$ for TLE: 78.64 $\pm$ 48.22 vs. XTLE: 24.58 $\pm$ 35.89, $p < 0.005$). This difference stands for net NSE concentration difference in TLE and XTLE during the whole video—EEG monitoring. The major difference was caused by the robust increase in NSE in TLE from 6 to 12 h as well as 12—24 h postictal time intervals (AUC$_{6-12h}$ for TLE: 25.6 $\pm$ 20.6 vs. XTLE: 7.8 $\pm$ 13.37, $p < 0.013$ and AUC$_{12-24h}$ for TLE: 36.67 $\pm$ 28.53 vs. XTLE: 6.69 $\pm$ 19.10, $p < 0.009$). The net proportional increase in NSE compared with baseline was 46.3 $\pm$ 41.2% in TLE and 12.2 $\pm$ 17.4% in XTLE ($p < 0.006$) during the 24 h monitoring. The time to reach maximum of circulating NSE was 10.2 $\pm$ 7.76 h for TLE and 7.88 $\pm$ 5.78 h for XTLE after index seizure ($p = 0.39$).

The mean number of seizures in the TLE group was 2.6 (range: 1—11). In the XTLE group one patient had 200 and another 100 seizures during the 24 h; excluding these patients the mean number in this group was 4.7 (range: 1—12). Five patients had only one brief seizure during the sampling period. The maximum change in the levels of NSE in these patients (mean: 6.4 μg/L; range: 4.9—7.9) was not statistically different from that in the other patients (mean:
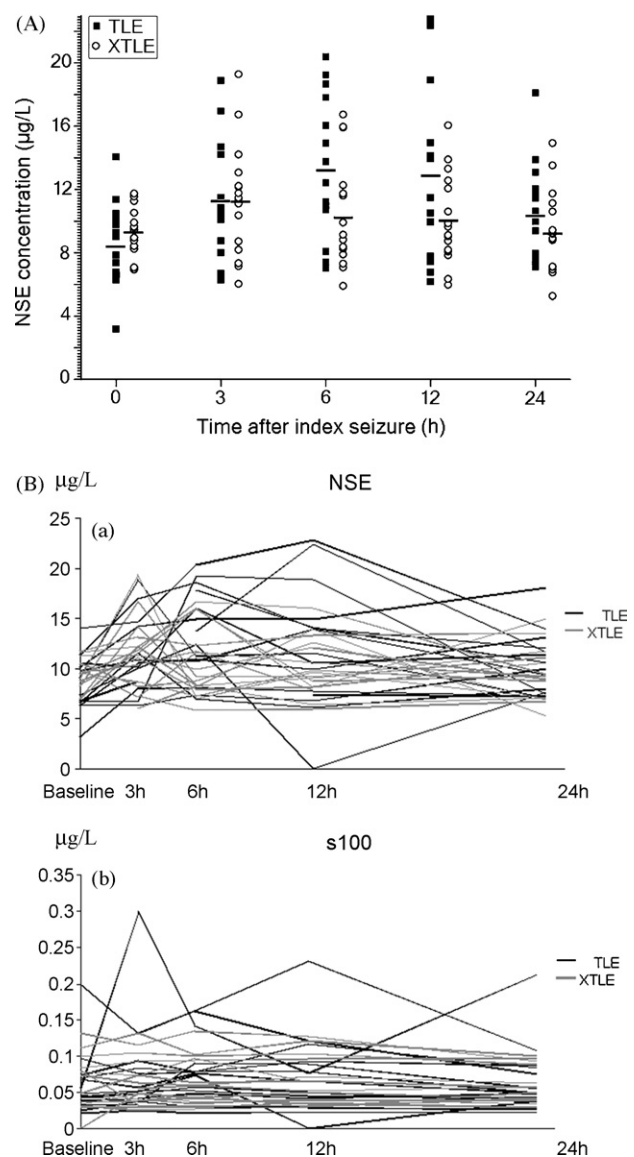

Figure 1 NSE concentrations (μg/L) in patients with temporal lobe (TLE) and extratemporal lobe epilepsy (XTLE) at each time point.

7.4 µg/L; range: 2.0—13.1). The mean seizure burden (seconds ± S.D.) in TLE was 259 ± 216 and in XTLE 2492 ± 7704. The median was 182 s in TLE and 260 s in XTLE. Five patients (three in the TLE, two in the XTLE group) had a secondarily generalized tonic-clonic seizure (SGTCS) as index seizure. Their mean NSE level at its highest was 11.39 µg/L at 6 h and the highest mean S-100 level was 0.135 µg/L at 3 h (n = 5). For partial seizures the mean level of NSE was 11.88 µg/L at 6 h and that of S-100 0.059 µg/L at 3 h (n = 26). The mean levels of NSE or S-100 were not significantly increased either within or between the subgroups of patients based on different etiologies of epilepsy.

Discussion

The present study suggests that self-limited seizures in TLE may be associated with an increase in serum levels of both NSE and S-100, and that seizures in chronic TLE may thus cause neuronal damage. The increased production of seizure-related NSE in TLE was observed at 3 h and it seemed to continue at least up to 24 h. The change in the serum levels of S-100 was less pronounced and of shorter duration. Peak levels of both NSE and S-100 in TLE-related seizures seemed to occur at 6 h postictally. The changes in serum levels of NSE and S-100 in XTLE-related seizures were not statistically significant, although the postictal levels showed an initial trend towards an increase. Thus, the risk of seizure-related neuronal damage may be different in TLE and in XTLE.

NSE in both serum and CSF has been previously studied in patients with status epilepticus or single brief seizures. There are indications that NSE levels can be increased by partial seizures, but more pronounced increase is seen after generalized or prolonged seizures (Pitkänen and Sutula, 2002). NSE has been increased in complex partial status epilepticus (CPSE), and high NSE levels reflect the long duration of CPSE (DeGiorgio et al., 1996, 1999). Four previous video—EEG studies with postictal measurements of either NSE or S-100 and with carefully localized seizures have been undertaken (Rabinowicz et al., 1996; Tumani et al., 1999; Leutmezer et al., 2002; Willert et al., 2004). In a study similar to ours (Tumani et al., 1999), NSE levels were increased in only a few of their patients, and mean levels of NSE were not significantly increased. Furthermore, the levels in the complex partial seizure (CPS) and secondarily generalized tonic-clonic seizure groups did not differ, but the NSE values peaked later in the SGTCS group (3 h vs. 1 h). Sixteen of their patients had TLE and five XTLE but they did not compare the results between two groups. The authors did not measure baseline levels but compared the postictal NSE levels with those of control subjects (Tumani et al., 1999). In a study which did not provide information on the localization of the seizure focus, elevated serum NSE levels were observed in altogether 34% of patients (both CPS and SGTCS) (Willert et al., 2004). Another study found increased serum levels of NSE both after single CPS (three of nine) and single SGTCS (four of four) (Rabinowicz et al., 1996). Few studies have measured S-100 after single seizures, but no postictal elevation of the levels has been noted (Büttner et al., 1999; Palmio et al., 2001; Leutmezer et al., 2002). In contrast to our findings, the only previous study of S-100 in TLE patients

in video—EEG did not find changes in S-100 (Leutmezer et al., 2002). As the number of patients was small (n = 10), different results might be explained by a random sampling effect and differences in the methods used for biochemical analyses.

Most of our patients had CPS as index seizure. Only three in the TLE and two in the XTLE group had SGTCS and their NSE levels do not explain the changes observed in these groups. However, S-100 levels were more increased at 3 h after SGTCS than after CPS (0.135 µg/L vs. 0.059 µg/L). The number of SGTCS patients was small, and more extensive studies are needed to determine whether S-100 levels increase significantly after SGTCS. Although many patients experienced recurrent seizures during the sampling period in our study, the number or duration of seizures did not explain the increased levels of NSE.

The magnitude of clinically significant increase in NSE or S-100 levels in terms of brain damage is not well established. The upper normal levels for NSE and S-100 vary in previous studies. Serum NSE concentration was 8.7 µg/L in a normal population (Casmiro et al., 2005), and the values of S-100 in healthy controls have been between 0.018 and 0.098 µg/L (Arts et al., 2006). The increase of these markers in epileptic patients might indicate a more subtle and transient injury. However, in patients with refractory epilepsy the cumulative effect of even transient brain injury associated with seizures might become clinically significant in the course of years.

Experimental models of epilepsy and the phenomenon of kindling have provided information on structural and functional changes in response to seizures. This plasticity in neural circuits may induce progressive damage but also resistance to additional damage (Sutula, 2004). Activation of mesial temporal structures is more prone to damage than are other areas of the brain. In experimental models status epilepticus causes neuronal loss in hippocampus (Ben-Ari, 1985; Pitkänen et al., 2002). Using the kindling model there is indication that also brief recurrent seizures can lead to cell loss and sprouting (Cavazos et al., 1994; Sutula et al., 1996). Supporting these observations, kindled seizures increased the concentration of NSE (Hansen et al., 1990).

Clinical and epidemiological studies have shown that a subset of patients with epilepsy has progressive features such as increasing seizure frequency and cognitive decline (Sutula, 2004). Progressive symptoms are particularly common in TLE (Pitkänen et al., 2002). The reviewed data on the effects of recurrent seizures on cognitive function have shown a slight but consistent relation between seizures and mental decline (Dodrill, 2002). Several MRI studies have shown an association between severity of hippocampal damage and the estimated total seizure number, seizure frequency, and duration of epilepsy (Van Paesschen et al., 1997; Salmenperä et al., 2001). A prospective MRI study with newly diagnosed focal epilepsy found that hippocampal damage occurs in individual patients during 2—3 years of follow-up. The patients who developed hippocampal volume decrease had seizures of longer duration and higher number before the diagnosis of epilepsy (Salmenperä et al., 2005). There is also evidence that the damage in TLE due to mesial temporal sclerosis may be more widespread and bilateral in patients with unilateral TLE (Araújo et al., 2006).

The evidence obtained from these studies suggests that chronic epilepsy and recurrent brief seizures can induce neuronal damage in individual patients with TLE. How-

ever, there is paucity of studies addressing XTLE and these features. Our present study adds to information on the consequences of seizures in TLE and XTLE. It is widely accepted that there is a strong correlation between hippocampal sclerosis (HS) and severity of epilepsy. Our study did not find significant changes in the subgroup of patients with HS or other etiological findings, possibly because of the limited number of patients in the different subgroups. More studies are needed to determine the etiological implications and also to follow-up patients with elevated markers of brain damage.

The markers of brain damage NSE and S-100 were elevated in TLE suggesting that brief seizures in TLE may induce neuronal damage, whereas there was no significant change in NSE or S-100 in patients with XTLE. It is accepted that all epilepsies do not cause brain damage nor are invariably progressive, and it would therefore be beneficial to be able to identify those patients who are at risk for damage. This information might have a reflection on treatment, i.e. proceeding to surgery or vagus nerve stimulation earlier in these TLE patients.

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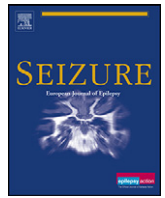
Cerebrospinal fluid tau as a marker of neuronal damage after epileptic seizure

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ABSTRACT

Purpose: Whether repeated brief seizures can cause neuronal damage is controversial. Cerebrospinal fluid (CSF) total tau (T-tau) and phosphorylated tau (P-tau) measurements have been suggested for the diagnosis of Alzheimer's disease, and T-tau may also be a marker of axonal damage and neuronal degeneration. We studied T-tau and P-tau levels and P-tau/T-tau ratio in CSF after epileptic seizures in order to determine whether they are increased after seizures.
Methods: A total of 54 patients with tonic–clonic or partial secondarily generalized seizures due to various etiologies were studied and CSF obtained within 48 h after the seizure.
Results: There were no statistical differences in the levels of T-tau ($p = 0.09$, ANOVA) or P-tau ($p = 0.60$) between different etiologic groups or controls. No patients with epilepsy of unknown origin had abnormal CSF T-tau whereas 11 patients with acute or remote symptomatic seizures had abnormal T-tau levels and the P-tau/T-tau ratio showed significant differences between the groups and controls ($p = 0.003$).
Conclusions: Epileptic seizures with unknown etiology did not increase CSF tau levels. Abnormal tau levels were associated with either acute or remote symptomatic seizures with known etiology. The presence of elevated CSF tau increases the probability of symptomatic cause in a patient with a seizure.

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1. Introduction

Clinical and experimental studies have shown that status epilepticus may induce neuronal damage, especially in the hippocampus. Whether repeated brief seizures can cause such neuronal damage is, however, controversial.[1,2] Markers of brain injury, such as neuron-specific enolase (NSE) or S-100b protein (S-100), have been measured in patients with seizures. Increased levels of NSE have been reported in a subset of epileptic patients.[3] However, in our previous study neither NSE nor S-100 was elevated in serum or cerebrospinal fluid (CSF) after a single tonic–clonic seizure in patients with a newly diagnosed seizure disorder.[4]

Tau is a microtubule-associated protein with a major role in normal microtubular function in axons. Tau is a phosphoprotein but abnormally high phosphorylation of tau protein decreases its ability to promote microtubular assembly. The equilibrium between phosphorylation and dephosphorylation modulates cytoskeletal stability and axonal morphology.[5] Tau protein is primarily localized in axons, and after brain parenchymal damage its release into CSF may increase.[6] Increased levels of CSF total tau (T-tau) and especially phosphorylated tau (P-tau) are frequently found in patients with Alzheimer's disease (AD) but also in patients with various neurological diseases, such as traumatic brain injury,[7] acute ischemic stroke,[8] and viral encephalitis, suggesting that T-tau CSF levels reflect the extent of axonal damage and neuronal degeneration.[9,10] CSF tau levels are very high in patients with Creuzfeldt–Jakob disease.[11]

To our knowledge, tau levels have not been previously measured after epileptic seizures. We hypothesized that CSF tau levels may be a marker of neuronal damage in patients suffering from epileptic seizures. Therefore, we studied T-tau and P-tau levels and P-tau/T-tau ratio in CSF after epileptic seizures due to various etiologies.

2. Materials and methods

The study was performed at the Emergency Department of Neurology, Tampere University Hospital, Finland. A total of 54 patients with tonic–clonic or partial secondarily generalized seizures were included in the study. The patients had either single or recurrent seizures (the median number of seizures was 1.5).

Nine patients had status epilepticus. There were 30 male and 24 female patients with a mean age of 48 years (range 16–88 years). All patients were fully informed of the risks and potential benefits of the CSF examination, which was performed to exclude CNS infection. Informed consent to participate in the study was obtained from each subject. The study protocol was approved by the Ethics Committee of Tampere University Hospital.

CSF tau increases with age.[12,13] The reference values for tau have been established for elderly patients (>60 years) in our laboratory.[14] Therefore, we obtained control samples from 31 younger (<60 years) patients with neurological symptoms (e.g. dizziness, headache) on whom lumbar puncture was performed to exclude neurological disease. Clinical examination or imaging studies (computed tomography (CT) or magnetic resonance imaging (MRI) of the head) revealed no pathological findings in control subjects. The mean age in the control group was 40 years (range 15–56 years).

The patients were divided into groups based on the underlying etiology of epileptic seizures. The seizures were caused by alcohol withdrawal in group 1 (ALCO, $n = 15$). In groups 2 and 3 the patients had diagnosis of epilepsy. Group 2 (SYMPT, $n = 16$) had remote symptomatic epilepsy and in group 3 (EPI, $n = 13$) the patients had either cryptogenic focal ($n = 10$) or idiopathic epilepsy ($n = 3$) with no related findings in brain CT or MRI. The etiology of remote symptomatic epilepsy (group 2) was tumor in the CNS ($n = 6$), post-stroke or post-traumatic epilepsy ($n = 7$), Alzheimer's disease ($n = 1$), multiple sclerosis ($n = 1$), and post-encephalitic epilepsy ($n = 1$). In group 4 (ACU, $n = 7$) seizures were caused by acute systemic illness (sepsis and hyponatremia) or acute CNS disorder (acute brain infarction, encephalitis, necrotizing encephalomyelitis). In addition to these four groups, three patients had only a single seizure of unknown etiology.

2.1. CSF sampling and biochemical analyses

The CSF samples were taken within 48 h (mean 14.4 h) after the seizure. The samples were stored at −70 °C prior to analysis. The CSF levels of T-tau and P-tau$_{(181P)}$ were measured by a commercial enzyme-linked immunosorbent assay, ELISA (Innogenetics, Ghent, Belgium) according to the manufacturer's protocol. The ELISA analyses were done blinded to the diagnostic group. Two patients in group 4 (ACU) and two in controls did not have enough CSF for P-tau measurement. P-tau/T-tau ratio was also calculated.

2.2. Statistical analysis

Because the parameters were not normally distributed non-parametric methods were used. Kruskal–Wallis ANOVA and Mann–Whitney U-test were used to compare concentrations of T-tau and P-tau. Frequency tables were compared with Pearson Chi-square test (in 2 × 5 tables, 4 degrees of freedom) and Fisher's exact test (post hoc comparisons in 2 × 2 tables, 1 degree of freedom) when appropriate. The correlations were calculated using Spearman's correlations. Statistical calculations were carried out using Statistica (ver. Win 5.1D, Statsoft Inc., Tulsa, OK, USA). Findings were considered statistically significant at p-values less than 0.05.

3. Results

The median T-tau level was 163.1 pg/mL and P-tau 39.6 pg/mL in all patients, and 143.5 pg/mL and 38.1 pg/mL in the controls (Fig. 1). The median P-tau/T-tau ratio was 0.23 in the patients and 0.26 in the controls. There were no statistical differences in T-tau ($p = 0.09$, ANOVA) or P-tau ($p = 0.6$, ANOVA) levels between different etiologic groups and controls (Table 1). The number of
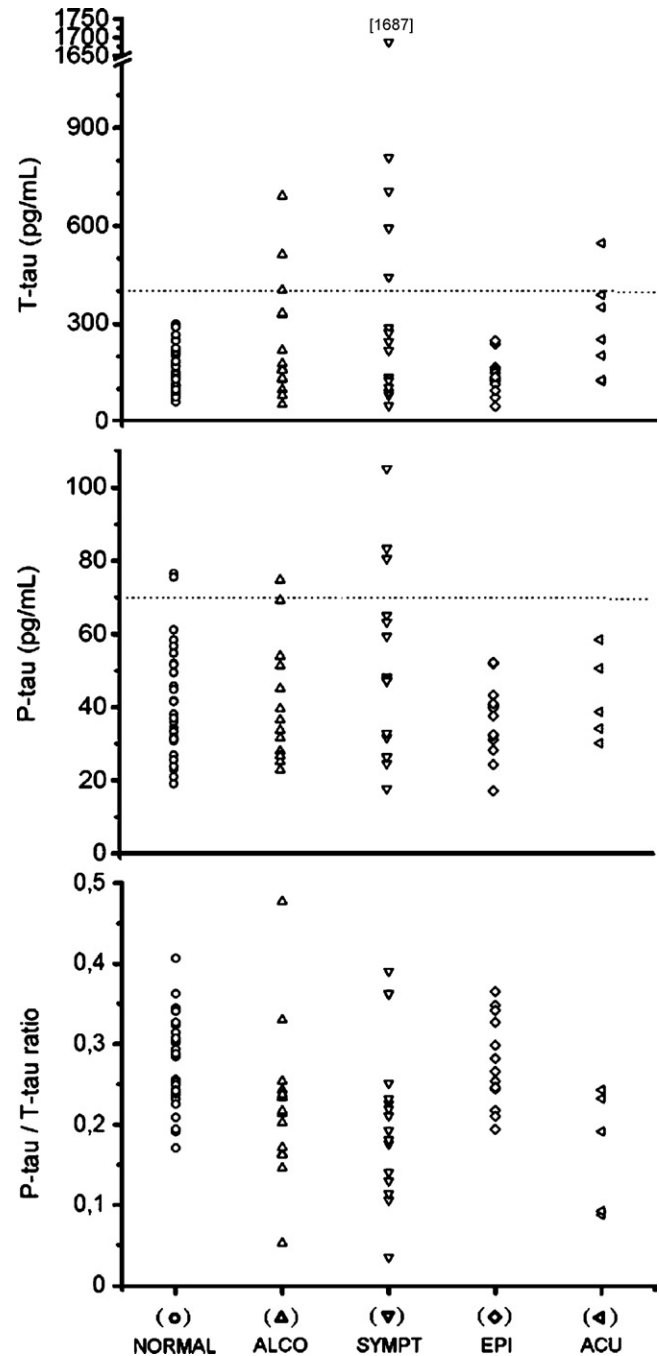

Fig. 1. The individual values of total tau (T-tau) and phosphorylated tau (P-tau) and P-tau/T-tau ratio in different patient groups and normal controls. ALCO, alcohol withdrawal; SYMPT, remote symptomatic epilepsy; EPI, cryptogenic focal, or idiopathic epilepsy; ACU, acute systemic or CNS illness. The horizontal lines represent the cutoff value for T-tau and P-tau (>60 years).

seizures, status epilepticus or the time interval between the seizures and CSF sample did not correlate with tau concentrations ($r = -0.17$ to 0.28, Spearman's correlations).

P-tau/T-tau ratio showed significant differences between the groups and controls ($p = 0.003$, ANOVA, Table 1). The controls differed from groups 1, 2 and 4 ($p = 0.010$–0.008 Mann–Whitney U-test) but there was no statistical difference between the age-matched controls and epilepsy group 3 ($p = 0.7$).

A concentration of T-tau above 400 pg/mL for patients over 60 years was considered abnormal as established in our laboratory.[14] Previous studies have suggested that the reference values for

Table 1

The median values of total tau (T-tau), phosphorylated tau (P-tau), and P-tau/T-tau ratio in different patient groups and controls.

	Group 1 (ALCO)	Group 2 (SYMPT)	Group 3 (EPI)	Group 4 (ACU)	Controls	*p*-Value ANOVA
No of patients	15	16	13	7	31	
The mean age (range)	49.5 (31–69)	60.0 (25–88)	35.9 (16–73)	45.3 (16–75)	40.3 (15–56)	
T-tau, pg/mL (quartiles)	163.1 (129.3–333.3)	259.1 (115.5–518.2)	133.4 (114.9–159.2)	251.9 (127.3–388.7)	143.5 (108.1–214.9)	0.090
P-tau, pg/mL (quartiles)	36.6 (26.8–53.9)	47.6 (29.1–64.2)	37.6 (31.1–41.0)	38.7 (34.2–50.6)	38.1 (31.3–51.6)	0.600
P-tau/T-tau ratio (quartiles)	0.217 (0.164–0.243)	0.202 (0.135–0.242)	0.266 (0.244–0.327)	0.191 (0.093–0.232)	0.256 (0.238–0.314)	0.003
The mean time interval[a] hours (range)	14.2 (4–24)	14.5 (3–48)	16.5 (6–48)	12.7 (5–24)		

Group 1 (ALCO), alcohol withdrawal; group 2 (SYMPT), remote symptomatic epilepsy; group 3 (EPI), cryptogenic focal, or idiopathic epilepsy; group 4 (ACU), acute systemic or CNS illness.

[a] Time interval between seizure and LP.

Table 2

Characteristics of the patients with elevated total tau (T-tau) levels.

Patients, age	Diagnosis, etiology of epilepsy	T-tau (pg/mL)	P-tau[a] (pg/mL)	P-tau/T-tau ratio
Group 1 (ALCO)				
No 1, 44 years	Alcoholic encephalopathy	692.0	36.6	0.053
No 2, 69 years	Wernicke's encephalopathy	512.3	74.8	0.146
No 3, 41 years	Alcohol withdrawal	403.4	69.2	0.172
Group 2 (SYMPT)				
No 4, 88 years	Alzheimer's disease	809.0	105.2	0.130
No 5, 62 years	Post-traumatic epilepsy	443.3	47.1	0.106
No 6, 86 years	Post-stroke epilepsy	1687.9	59.4	0.035
No 7, 32 years	Post-encephalitic epilepsy	593.1	83.4	0.141
No 8, 66 years	Large inoperable pituitary adenoma	706.2	80.6	0.114
Group 4 (ACU)				
No 9, 16 years	Viral encephalitis	388.7	34.2	0.088
No 10, 74 years	Viral encephalitis	546.6	50.6	0.093
No 11, 17 years	Necrotizing encephalomyelitis	350.2	ND	ND

Group 1 (ALCO), alcohol withdrawal; group 2 (SYMPT), remote symptomatic epilepsy; group 4 (ACU), acute systemic or CNS illness.

[a] Phosphorylated tau.

younger patients are lower.[12,13] We used 300 pg/mL as reference value for patients below 60 years. However, we found no differences in the mean levels of CSF T-tau or P-tau between age-matched controls and patients with epilepsy group 3.

There was statistical difference in the frequency of abnormal T-tau levels between the groups studied (*p* = 0.002, Pearson Chi-square). None of the patients with epilepsy of unknown origin (group 3) or the controls had abnormal levels of T-tau. Three patients with a single seizure had also normal levels of T-tau. However, a total of 11 patients (20%) in other groups had abnormal T-tau levels. The characteristics of these patients are shown in Table 2.

In group 1 (ALCO) 20% had abnormal tau concentrations, and two of them showed also brain atrophy in MRI. In group 2 (SYMPT) tau was abnormal in 31%, and all of them showed brain pathology in MRI; two with brain atrophy, one with old contusion and brain atrophy, one with large basal expansion (pituitary adenoma), and one with hippocampal atrophy after encephalitis. In group 4 (ACU) 43% had abnormal tau concentrations, two patients had acute encephalitis and one had necrotizing encephalomyelitis due to intrathecal cytarabine treatment for Burkitt's lymphoma. The controls differed from groups with either acute or remote symptomatic seizures (groups 1, 2 and 4; *p* = 0.03–0.003, Fisher's exact test) but not from those with epileptic seizures with unknown etiology (group 3, NS). When groups 2 (SYMPT) and 3 (EPI), i.e. epilepsy without acute illness, were compared, there was also statistical difference in the number of abnormal T-tau (*p* = 0.04, Fisher's exact test).

4. Discussion

Our results showed that CSF tau levels were within the normal range in patients with idiopathic or cryptogenic epilepsy, and increased levels were only found in patients with either acute or remote symptomatic seizures.

To our knowledge seizure related changes of CSF tau have not been previously studied in patients with different etiologies of epilepsy. A case report based on a single patient found a transient elevation of CSF tau levels in one patient measured 4 days after the seizure.[15] CSF tau showed an increase at 2–3 days after acute stroke[8,16] but in traumatic brain damage and brain injury after aortic surgery the increase in tau levels started earlier, during the first day.[7,17,18] The time interval between LP and seizure varied (mean 14.4 h) in our patients but no correlation between tau levels and the time interval was observed.

Elevated CSF T-tau levels have been consistently found in AD and have been suggested to be useful in differentiating AD from normal ageing and depression.[19] However, an overlap exists with other neurodegenerative disorders or conditions with brain parenchymal damage.[9,20] P-tau is considered to reflect the phosphorylation state of tau, being a more direct marker for AD. Consistent with this, in patients with ischemic stroke CSF T-tau but not P-tau levels were increased and showed correlation with the size of the infarct, indicating that CSF T-tau reflects the degree of neuronal damage.[8,16] Also in Creuzfeldt–Jakob disease marked elevation of T-tau but not P-tau levels has been reported.[11] In Kapaki's study sensitivity and specificity of P-tau/T-tau ratio were better in the discrimination of AD versus controls than P-tau alone.[20] The best cutoff level for this ratio was ≥0.169. Most of our patients with abnormal T-tau levels had quite low P-tau and also low P-tau/T-tau ratio indicating rather non-specific neurodegenerative disorder or axonal damage.

CSF T-tau has been studied in alcohol related cognitive disorders (ARCD) in attempt to discriminate AD from other dementias using tau measurements. Tau pathology has been found

in chronic alcoholics with thiamine deficiency and degeneration of nucleus basalis may develop through formation of neurofibrillary tangles.[21] This may account for the higher tau values for the ARCD patients compared with the controls[22] or increased tau levels seen in patients with alcohol dementia.[23] In contrast to this, CSF tau was within normal levels in demented and non-demented alcoholics compared with AD patients and controls.[24] In our patients with alcohol withdrawal seizures (group 1) 20% had elevated T-tau levels, two with known alcoholic encephalopathy.

Increased levels of tau have also been reported in patients with cerebral infections. In one study 18% of HIV infected patients had increased CSF tau levels, but of those who had cerebral infections and necrosis elevated tau levels were found in 35%.[25] In Süssmuth's study, patients with viral encephalitis and bacterial meningitis with meningoencephalopathic complications had elevated tau levels.[10] The authors suggested that tau may be a marker for parenchymal involvement. Consistent with this, three patients in group 4 (ACU) had abnormal levels of tau, two with acute viral encephalitis, one with necrotizing encephalopathy.

Other biomarkers, mainly NSE and S-100, have been used to assess possible brain damage after seizure. Several studies have reported raised serum and CSF NSE levels after brief seizures as well as after status epilepticus,[3,26] but no postictal elevation of S-100 after single seizures has been observed.[4,27,28] However, cisternal S-100 as well as NSE levels ipsilateral to the site of seizure onset were elevated interictally in intractable temporal lobe epilepsy.[29] The reduction of endogenous tau levels protected mice against excitotoxicity in an Alzheimer's disease mouse model,[30] indicating that tau may mediate excitotoxicity. Therefore high CSF tau levels after seizure could mark a poorer prognosis. In this study, the seizures without additional pathology were not associated with high tau levels. Furthermore, the number of seizures or status epilepticus did not correlate with the levels of tau.

We conclude that abnormal CSF tau levels may be found in patients with seizures. However, acute seizure itself does not seem to lead to the elevation of CSF tau. The presence of increased CSF tau in a patient with epileptic seizure increases the probability of symptomatic cause.

Acknowledgement

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