

Sustained Elevation of Kynurenic Acid in the Cerebrospinal Fluid of Patients with Herpes Simplex Virus Type 1 Encephalitis

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ABSTRACT: Herpes simplex virus (HSV) type 1 encephalitis (HSE) is a viral infectious disease with commonly occurring neurodegeneration and neurological/cognitive long-term sequelae. Kynurenic acid (KYNA) is a neuroactive tryptophan metabolite, which is elevated in the cerebrospinal fluid (CSF) during viral infection as a result of immune activation. The aim of the study was to investigate the role of endogenous brain KYNA for the long-term outcome of the disease. CSF KYNA concentration was analyzed in 25 HSE patients along the course of the disease and compared with that of 25 age-matched healthy volunteers. Within 3 weeks of admission CSF KYNA of HSE patients was markedly elevated (median 33.6 nM) compared to healthy volunteers (median 1.45 nM). Following a decline observed after 1–2 months, levels of CSF KYNA were elevated more than 1 year after admission (median 3.4 nM range: 1–9 years). A negative correlation was found between initial CSF KYNA concentrations and severity of the long-term sequelae. This study shows a marked elevation in CSF KYNA from patients with HSE, most pronounced during the acute phase of the disease and slowly declining along the recovery. We propose that brain KYNA might potentially protect against neurodegeneration while causing a long-lasting loss in cognitive function associated with the disease.

KEYWORDS: Tryptophan, NMDA hypofunction, HSE, neurodegeneration, cognition

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Introduction

Herpes simplex virus (HSV) encephalitis (HSE) is the most frequent cause of sporadic necrotizing encephalitis in adults.^{1–3} Despite pharmacological treatment the associated mortality rate is still high, approximately 20% and permanent disability, particularly cognitive and memory impairment is common.^{4–6} The acute manifestation of HSE is not different from other encephalitis, and symptoms, ranging from mild to severe,^{7,8} include fever, headache, personality and behavioral changes, and focal neurological signs. Seizures during the acute stage are more common than in other encephalitis and occur in

33–67% of HSE patients.^{9–11} The long-term symptoms, occurring partly as a result of neurodegeneration¹² varies from those seen at onset to non-febrile symptoms such as memory loss, cognitive deterioration and neurological/psychiatric manifestations (eg, depression, agony and sleeping disorder).^{9,13,14} Despite acyclovir treatment approximately 30–62% of the patients suffer from moderate to severe long-lasting sequelae, that affects both functioning and personality with pronounced impairment in quality of life.^{10,11,15} Neuropsychiatric sequelae are known to occur after HSE and the most common disabling symptom is cognitive dysfunction.^{4,16} The pathogenesis



of both HSE and the neuropsychiatric sequelae is unclear, both with regard to the primary infection and to the reactivation. There is no evidence that antibodies may protect against clinical manifestations of HSV and several studies have demonstrated that neutralizing specific antibodies do not provide protection against HSV infection or disease.¹⁷ Immune compromised patients with defects in cell mediated immunity experience more severe and more extensive HSV infections than those with deficits in humoral immunity.^{18–20} Increased knowledge of the mechanism behind the symptoms is crucial for improved treatment of the disease.

It is known that immune activation induces the kynurenine pathway of tryptophan degradation.^{21–23} This pathway is not only an important regulator of the innate and adaptive immune response²⁴ but is also responsible for the biosynthesis of several neuroactive compounds. Kynurenic acid (KYNA) is an end-metabolite in one branch of this pathway. The compound is synthesized by astrocytes in the brain and acts an antagonist at both the glycine site of the N-methyl-D-aspartate (NMDA) receptor^{25–27} and at the cholinergic $\alpha 7$ nicotine receptor.²⁸ Apart from being neuroprotective and anticonvulsant,²⁹ KYNA is also implicated in cognitive functions. Thus, elevated brain KYNA in rodents is associated with deficits in prepulse inhibition,³⁰ spatial and contextual learning and memory,^{31,32} and impaired cognitive flexibility.^{33,23} Notably, elevated levels of KYNA have been detected in several diseases characterized by cognitive impairments,³⁴ eg, tick-borne encephalitis,³⁵ encephalitis caused by human immunodeficiency virus 1,³⁶ schizophrenia^{37–40} and bipolar disorder.⁴¹

In the present study, CSF KYNA was analyzed concomitant with symptom manifestation along the course of HSE, hereby enabling assessment of the role of the compound in the pathophysiology of the disease.

Materials and methods

Ethics. The work described in the present study was carried out in accordance with “The code of ethics of the world medical association (declaration of Helsinki) for experiments including humans: “<http://www.wma.net/en/30publications/10policies/b3/>.” The Ethical Committee on Human Investigations of the Faculty of Medicine, Karolinska Institutet, and the Regional Ethical Review Board in Stockholm approved the study. All participants provided written informed consent.

Subjects. KYNA was measured in CSF samples collected from 11 men and 14 women with herpes simplex type 1 encephalitis (Table 1). All patients in the study were retrospectively included and treated at the Department of Infectious Diseases at Danderyds Hospital, Stockholm from 1973–1996. CSF samples were obtained at enrollment, and all patients had clinical signs of encephalitis with CSF pleocytosis ($>5 \times 10^6$ cells/L). 25 age-matched healthy volunteers, 11 men and 14 women, were used as controls (Table 2). Controls were free of medication for at least 1 month and free from any form of substance abuse.

CSF levels of KYNA from these individuals have been previously published.^{39–41}

14 patients were diagnosed by the demonstration of HSV specific intrathecal antibody synthesis- indirect immunoassays by IgG in CSF and serum samples.¹ 5 patients were diagnosed by demonstration of viral antigen in specimens obtained by brain biopsy.⁴² 1 patient was diagnosed by virus isolation from the throat and another by virus isolation from the CSF and 4 patients were diagnosed by IgM and IgG in sera.⁴³ It was not possible to establish if the patients suffered from a primary or recurrent HSV infection due to lack of clinical history and diagnostic evidence. Co-infections with HSE and staphylococci septicemia were observed in 2 patients. One patient with HSE had alcohol abuse. 3 patients died during the first 3 weeks after admission, from lung edema, emboli of the heart and brain edema, respectively. Another patient died at day 54 after admission with brain edema. All patients were culture negative for bacteria and fungi in CSF. Demographic and clinical data were collected from medical records.

There are no specific clinical symptoms in HSE that differ from other encephalitis.⁷ During the acute onset of HSE, almost 100% of the patients presented fever, headache, personality and behavioral changes, including psychosis, and focal neurological signs (see Table 3). Half of the patients experience prodromal symptoms less than a week before onset of encephalitis symptoms. Patients in this study experienced symptoms of encephalitis for an average of 5 days before they were admitted to the hospital (mean 5 days, range 1–22 days). At enrollment all patients with HSE underwent a clinical examination and their symptoms were classified as “mild,” “moderate,” or “severe” encephalitis. The mild form was defined as a disease with predominantly meningeal symptoms, including fever, headache, rigidity of the neck, and nausea. The moderate form was defined as a disease with monofocal symptoms of the CNS and/or moderate diffuse brain dysfunction (in addition to symptoms seen in the mild form), whereas the severe form additionally included multifocal symptoms of the CNS and/or severe diffuse brain dysfunction.

The clinical outcome at long-term follow up was evaluated and graded as mild sequelae (minor impediment to activities of daily living), moderate sequelae (considerable impediment to activities of daily living), or severe sequelae (under institutional care).

Pharmacological treatment. All patients were given antiviral agents at admission: 11 patients were treated with acyclovir (10 mg/kg \times 3 intravenously 10–14 days), 10 patients were treated with vidarabine (600–700 mg/day for 10 days), and 4 patients were treated with cytarabine (300 mg/day for 7 days). 19 patients received dexamethasone (4 mg \times 4 for 3 days).

16 patients received phenytoin to prevent seizures, three of them received additional treatment with carbamazepine or diazepam.

Cerebrospinal fluid sampling. CSF samples were collected within 4 different time frames with regard to admission

Table 1. Demographic characteristics, baseline clinical, and laboratory findings for 25 patients with herpes simplex encephalitis.

DIAGNOSIS	AGE AT ONSET	SEX	SYMPTOM CLASSIFICATION AT ONSET	KYNA 0–3 WEEKS	KYNA 1–2 MONTHS	KYNA 6–12 MONTHS	KYNA >1 YEAR	TREATMENT AT ADMISSION	ANTIEPILEPTIC TREATMENT	SEQUELE CLASSIFICATION AFTER ONE YEAR
HSE*	17	male	severe	3.39	–	–	1.11	acyclovir/ dexamethasone	–	fully recovered
HSE	62	male	moderate	302.52	–	8.57	8.35	acyclovir	phenytoin	moderate
HSE	21	female	moderate	–	–	2.82	2.69	cytarabin/ dexamethasone	phenytoin	moderate
HSE	35	female	severe	31.05	11.31	–	10.67	acyclovir	phenytoin	moderate
HSE	70	male	moderate	19.26	6.11	–	5.72	acyclovir/ dexamethasone	–	n/a
HSE	32	female	moderate	–	–	–	3.61	vidarabin/ dexamethasone	–	n/a
HSE	51	male	moderate	1.73	5.37	–	–	acyclovir/ dexamethasone	phenytoin	n/a
HSE	59	female	n/a	–	–	–	3.18	cytarabin/ dexamethasone	–	moderate
HSE	61	female	moderate	26.44	11.06	–	45.56	acyclovir/ dexamethasone	phenytoin. diazepam	severe
HSE	71	male	severe	26.78	8.31	–	1.59	acyclovir/ dexamethasone	phenytoin	severe
HSE	27	male	mild	52.27	–	2.32	1.41	acyclovir/ dexamethasone	–	n/a
HSE	76	male	moderate	89.68	53.43	–	4.21	acyclovir	–	n/a
HSE	37	female	severe	–	–	–	3.11	cytarabin/ dexamethasone	phenytoin. carbamazepine	moderate
HSE	35	male	moderate	–	–	1.22	1.54	vidarabin	–	severe
HSE	57	male	moderate	145.47	5.29	–	1.25	vidarabin/ dexamethasone	phenytoin	moderate
HSE	34	female	severe	46.05	10.61	–	7.39	cytarabin/ dexamethasone	phenytoin	n/a
HSE	61	male	severe	–	–	–	2.94	vidarabin/ dexamethasone	phenytoin	severe
HSE	54	female	severe	6.67	1.98	–	5.21	acyclovir/ dexamethasone	phenytoin	severe
HSE	63	female	moderate	115.71	26.64	–	5.34	acyclovir/ dexamethasone	phenytoin. carbamazepine	moderate
HSE	30	female	moderate	19.35	–	–	1.97	vidarabin/ dexamethasone	phenytoin	severe
HSE	30	male	mild	98.07	–	13.07	3.76	vidarabin/ dexamethasone	–	severe
HSE†	62	female	severe	71.66	–	–	–	vidarabin/ dexamethasone	phenytoin	n/a
HSE†	75	female	moderate	51.56	–	–	–	vidarabin	–	n/a
HSE†	71	female	severe	22.07	–	–	–	vidarabin	phenytoin	n/a
HSE†	16	female	moderate	33.63	–	–	–	vidarabin/ dexamethasone	phenytoin	n/a

*Patient almost fully recovered 3 weeks after admission.

†Patient died within 2 months after admission.

1) 0–3 weeks (n = 19), 2) 1–2 months (n = 9), 3) 6–12 months (n = 6), and 4) >1 year (n = 19). CSF was obtained by lumbar puncture (L4–L5) in a standardized manner. The lumbar punctures were performed with subjects in a lateral recumbent position. The third 1 mL portion of the CSF was frozen in 2

portions (0.5 mL each) at –25° C and was subsequently used for biochemical analysis. All CSF samples used in this study were collected between years 1973 and 1996.

Analysis of CSF KYNA. KYNA is a stable compound and is not degraded even by repeated thawing.⁴⁴ We performed

Table 2. Healthy volunteers.

	FEMALE	MALE	ALL
N	14	11	25
Median age (range)	30 (25–73)	38 (31–51)	32 (25–73)
Median KYNA, nM (p25–p75)	2.33 (1.42–3.20)	1.29 (1.11–1.33)	1.45 (1.16–2.61)

the analysis of KYNA using an isocratic reversed-phase high-performance liquid chromatography (HPLC) system, including a dual piston, high liquid delivery pump (Bischoff, Leonberg, Germany), a ReproSil-Pur C18 column (4 × 150 mm, Dr Maisch GmbH, Ammerbuch, Germany) and a fluorescence detector (Jasco Ltd, Hachioji City, Japan) with an excitation wavelength of 344 nm and an emission wavelength of 398 nm (18 nm bandwidth) essentially as previously described.⁴⁰ A mobile phase of 50 mM sodium acetate pH 6.20 (adjusted with acetic acid) and 7.0% acetonitrile was pumped through the reversed-phase column at a flow rate of 0.5 mL/min. Samples (diluted in saline) of 30 µL were manually injected (Rheodyne, Rhonert Park, CA, USA). Zinc acetate (0.5 M, not pH adjusted) was delivered after the column by a peristaltic pump (P-500, Pharmacia, Uppsala, Sweden) at a flow rate of 10 mL/h. Signals from the fluorescence detector were transferred to a computer for analysis with Datalys Azur (version 4.6.0.0; <http://datalys.net>). The retention time of KYNA was about 7 min. The sensitivity of the system was verified by analysis of standard mixture of KYNA with concentrations from 0.5–30 nM, which resulted in a linear standard plot. The samples were analyzed in singles. Some samples were analyzed in duplicates, and the intra-individual variation was less than 5%.

Statistical analysis. Results are presented as medians and inter-quartile range (P25–P75). The Mann-Whitney U test was used to analyze group differences regarding CSF levels of KYNA and Spearman rank order correlation coefficient was used to measure the association between KYNA levels and age. $P < 0.05$ was considered statistically significant.

Results

Analysis of CSF from age- and gender matched healthy volunteers show KYNA levels within a narrow concentration span at the same magnitude as those previously found in healthy volunteers (Table 2).^{36,37} CSF KYNA of HSE patients, obtained within 3 weeks of admission, was significantly elevated (median 33.6 nM (19.4–89.7), $n = 19$; Fig. 1) compared to healthy volunteers (median 1.45 nM (1.23–2.46), $n = 25$, $P < 0.0001$). The variation in CSF KYNA concentration in HSE patients within this time interval was very large, with a minimum and maximum value of 1.73 and 302 nM, respectively (Fig. 1). The initially high concentration of CSF KYNA progressively declined within 2 months. However, levels of CSF KYNA were significantly elevated more than 1 year after admission (median 3.40 (1.69–5.63),

$n = 18$, range: 1–9 years; $P < 0.0013$) compared to healthy volunteers (Fig. 1).

No significant differences in CSF KYNA levels were observed, at any time period, between patients that were subdivided into groups based on symptom severity at admission (Fig. 1). However, long-term symptoms were found to be associated with initial levels of CSF KYNA at admission. Thus, patients with severe sequelae ($n = 5$) displayed lower levels of CSF KYNA within 3 weeks at admission compared to the group of moderate sequelae ($n = 4$; Fig. 2).

Notably, 1 subject with staphylococci septicemia during admission displayed the highest detected level of CSF KYNA compared to other patients. We could not find any differences in CSF KYNA levels between genders neither within 3 weeks after admission (female median 32.2 nM (21.4–56.6), $n = 10$; male median 52.3 nM (11.3–121), $n = 9$, $P = 0.66$), nor at any other point of time. Furthermore, KYNA levels did not associate with consciousness or seizures (results not shown).

Discussion

The present longitudinal study allows an estimation of CSF KYNA in HSE patients along the course of infection. Our results show that CSF KYNA varies along different stages of the disease and that very high CSF levels, compared to healthy volunteers, are observed during the first 3 weeks of infections. Although CSF KYNA levels started to decline in most HSE patients after 1 to 2 months, levels were still significantly above those of healthy controls more than 1 year after admission.

The activity of the kynurenine pathway is critically regulated by the rate-limiting enzymes indoleamine 2,3-dioxygenase (IDO) and tryptophan-dioxygenase (TDO). One of these enzymes, IDO, is potentially induced by pro-inflammatory

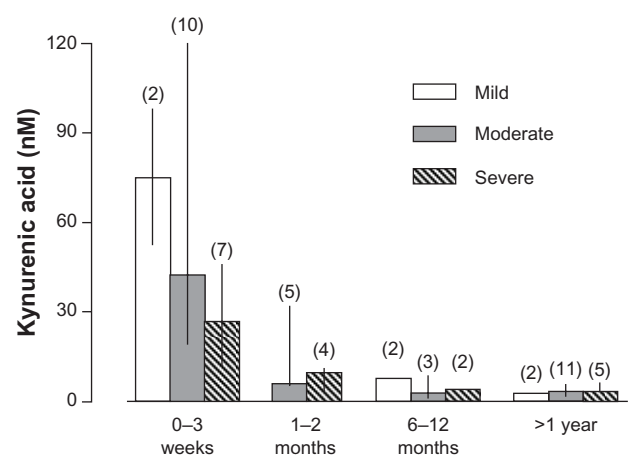


Figure 1. CSF kynurenic acid at different time points after admission in HSE patients with mild, moderate or severe symptoms at onset. The number of patients are indicated within brackets. Values are given as medians except for columns with only 2 samples where means are shown. Error bars show 25th and 75th percentiles.

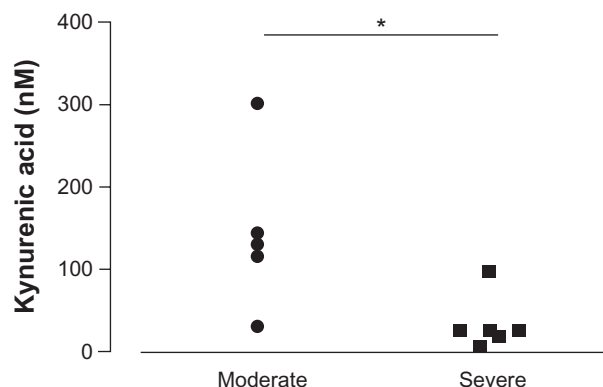


Figure 2. Relationship between initial CSF KYNA levels (obtained within 3 weeks after admission) and clinical outcome at long-term follow up (one year after initial admission). Error bars show 25th and 75th percentiles (* $P = 0.018$, Mann-Whitney U-test).

cytokines like interferon (IFN)- γ .^{45,23} In this context, previous studies have shown a significant elevation in CSF IFN- γ in patients with HSE.^{46,47} Notably, HSE in mice is associated with an increased activity of IDO in the spinal cord.⁴⁸ Thus, the presently observed elevation of CSF KYNA in patients with HSE may be causally related to an activation of cytokines; eg, an excess of brain IFN- γ , that subsequently induces IDO of the kynurenine pathway.

The significance of a facilitated tryptophan degradation via the kynurenine pathway as a result of an immune activation is unclear and it is a challenging question whether activation of the kynurenine pathway is forcing up the malignity of an infection, or alternatively, serves to dampen infections and prevent its consequences in terms of neuroprotection. The break-down of tryptophan by the kynurenine pathway generates several metabolites that in a complex manner modify the responsiveness of the immune system to inflammation and infection.⁴⁹ Thus, a decreased availability of tryptophan, eg, as a result of increased activity of the kynurenine pathway, may serve as a defense mechanism of the host by reducing the local supply of this essential amino acid to intracellular pathogens.⁵⁰ However, several metabolites of the kynurenine pathway display immunotolerant properties, including a reduction in pro-inflammatory T cells (Th1, Th17) or enhancement of anti-inflammatory T cells.^{51,52} In particular, IDO activation and the production of down-stream kynurenine metabolites is associated with development regulatory T cells,⁵³ which may restrict an ongoing specific antiviral immune response. Likewise, the inhibition of IDO may thus improve the efficiency of a cytotoxic T cells response in HIV-1 encephalitis.⁵⁴ Yet, with regard to the presently observed activation of the kynurenine pathway in HSE, the detailed immunological significance remains obscure.

KYNA may also directly affect neurodegeneration, which is frequently seen in HSE. Several metabolites of the kynurenine pathway, including KYNA and quinolinic acid,

are neuroactive, blocking and stimulating the glutamatergic NMDA receptor, respectively. The neuroprotective action of KYNA is well described,⁴⁹ and with regard to HSE, such an effect would principally serve to counteract neurodegeneration. In the present study, we found a correlation between clinical outcome in the long-term follow up and levels of CSF KYNA at admission. Thus, patients with a moderate sequelae as observed in follow up (see Methods) showed significantly higher CSF KYNA at admission compared to patients with a severe sequelae. This finding is the first to offer clinical support for a neuroprotective effect of endogenous KYNA in a condition of brain inflammation. In contrast to KYNA, quinolinic acid displays neurotoxic and convulsive properties.^{55,56,29} This relatively weak NMDA-receptor agonist is shown to be increased in the spinal cord of HSV-1 infected mice with encephalitis.^{57,48} However, in the presence of elevated levels endogenous KYNA, the neurotoxic actions of quinolinic acid would be less pronounced. Indeed, KYNA has previously been shown to critically control the vulnerability of mice brain striatal neurons to quinolinic acid.⁵⁸

A large body of previous studies implicates brain KYNA in psychotic disorders.^{36,59,37,23,40,60} The results of the present study strongly support this view; although many patients had alterations in consciousness at admission that may have masked psychiatric symptoms, psychotic behavior was commonly seen in line with observations of Whitley.⁶ Brain KYNA is also suggested to account for the cognitive deficits seen following other brain infections, such as HIV-1 encephalitis³⁶ or tick-borne encephalitis (TBE).³⁵ A majority of the surviving HSE patients develop chronic cognitive deficits, probably related to the loss of tissue in the temporal lobes. Numerous studies show an intimate relation between glutamatergic/cholinergic activity and cognitive functions.^{61,62} In consonance, increased levels of brain KYNA have recently been shown to impair spatial working memory³⁰ and cognitive flexibility³² in the rat. Similarly, a reduction in endogenous KYNA enhances cognitive behavior.⁶³ The elevated levels of KYNA observed in the present study may thus relate to psychotic symptoms during the acute phase and may partly account for the long-term cognitive deficits of the disease. Altogether, KYNA may paradoxically affect cognition in HSE patients in 2 opposite ways; the neuroprotective action of the compound during the acute phase of the disease may serve to prevent for general long-term sequelae, whereas chronic elevation of the compound after infection recovery may generate persistent cognitive disabilities.

A confound of the present study is the medication of the HSE patients. In order to minimize brain swelling and epileptic seizures most patients received dexamethasone and/or phenytoin, drugs interfering directly with the enzymes regulating the formation of KYNA. Glucocorticoids stimulate the activity of the IDO/TDO⁶⁴⁻⁶⁶ and anti-epileptic drugs like phenytoin or carbamazepine have been shown to augment



Table 3. Clinical findings and symptoms in patients with HSE at admission.

TOTAL NUMBER OF PATIENTS	25
Gender M/W	11/14
Median age (range)	54 (16–76)
Days from neurological onset to first LP	4.3 (range 1–19)
Headache	19 (76%)
Fever	24 (96%)
Myalgia	6 (24%)
Alteration of consciousness	24 (96%)
Consciousness at admission: Lethargy	13 (52%)
Consciousness at admission: Semicoma	9 (36%)
Consciousness at admission: Coma	2 (8%)
Personality change	23 (92%)
Seizures both focal and general	4 (16%)
Focal	5 (20%)
General	10 (40%)
Cranial nerve defects	9 (36%)
Visual field loss	1 (4%)
Vomiting	12 (48%)
Somnolence	22 (88%)
Dysphasia	20 (80%)
Dysesthesia	1 (4%)
Autonomic dysfunction	7 (28%)
Ataxia	4 (16%)
Hemiparesis	4 (16%)
Memory loss	20 (80%)
Confusion	24 (96%)
Hallucination	7 (28%)
Fatigue	24 (96%)

the activity of kynurenine aminotransferase (KAT) thereby increasing the synthesis of brain KYNA.⁶⁷ However, since dexamethasone treatment only lasted for 3 days in the majority of patients and the biological activity in the tissues is estimated to last another 2 days, the impact on KYNA levels after the first week may be limited. Moreover, there was no statistical difference in KYNA levels between HSE patients receiving glucocorticoids and those who did not. The information on how long time the patients received phenytoin is lacking, but there was no statistical difference with regard to KYNA levels between patients that received phenytoin and those who did not. All patients received acyclovir and the incorporation of defective thymidine residues into the viral genome utilizing viral thymidine kinase is a well established mechanism to reduce herpes virus replication. Although few serious side effects have been reported for acyclovir, this drug has also been suggested to inhibit IDO/TDO in rat brain.^{68,69} If anything,

such an effect of acyclovir should reduce the formation of KYNA. Taken together, a pharmacological contribution to the changes in kynurenic acid levels cannot be fully excluded, in particular a contribution of phenytoin to the early increase in CSF KYNA.

In summary, the results of the present study describe a marked elevation in CSF KYNA from patients with HSE, most pronounced during the acute phase of the disease and generally declining along the recovery. With regard to severe encephalitis, brain KYNA may serve as a double-edged sword, showing an important neuroprotective action preferentially during the acute phase of the disease, along with a long-term cognitive impairment most visible during and after recovery.

Author Contributions

Conceived and designed the experiments: AA, EF, BS. Analyzed the data: AA, EF, JS, LS. Wrote the first draft of the manuscript: AA, EF, JS. Contributed to the writing of the manuscript: GE, LS, EJ, MS. Agree with manuscript results and conclusions: AA, EF, JS, EJ, MS, LS, BS, GE. Jointly developed the structure and arguments for the paper: AA, EF, JS, LS, GE. Made critical revisions and approved final version: AA, EF, JS, EJ, MS, LS, BS, GE. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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