Neuroprotective Effect of Excitatory Amino Acid Antagonist Kynurenic Acid in Experimental Bacterial Meningitis

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Sustained high-level exposure to glutamate, an excitatory amino acid neurotransmitter, leads to neuronal death. Kynurenic acid attenuates the toxic effects of glutamate by inhibition of neuronal excitatory amino acid receptors, including the N-methyl-D-aspartate subtype. To evaluate the role of glutamate in causing neuronal injury in a rat model of meningitis due to group B streptococci, animals were treated with kynurenic acid (300 mg/kg subcutaneously once daily) or saline beginning at the time of infection. Histopathologic examination after 24–72 h showed two distinct forms of neuronal injury, areas of neuronal necrosis in the cortex and in injury of dentate granule cells in the hippocampus. Animals treated with kynurenic acid showed significantly less neuronal injury (P < .03) in the cortex and the hippocampus than did untreated controls. These results suggest an important contribution of glutamate to neurotoxicity in this animal model of neonatal meningitis.

Bacterial meningitis remains an important clinical problem and continues to result in significant brain injury in many patients, despite the use of highly active antibiotics [1]. The prognosis for the disease is particularly poor in neonates, as reflected by high mortality rates of 20%–30% and substantial morbidity in survivors [2]. In a study of neonates and young infants surviving group B streptococcal (GBS) meningitis, about one-third of patients were found to have major neurologic sequelae [3].

To investigate new therapeutic strategies to reduce neuronal injury during meningitis, we have developed a rat model of neonatal meningitis due to GBS [4]. The central nervous system histopathology in our model closely resembles that described in humans dying from the disease [5]. Acute inflammation in the subarachnoid space and ventricles, inflammatory involvement of the cerebral vasculature, widespread cortical neuronal injury, infarcts, and reactive astrocytosis are histopathologic hallmarks both in the human disease and in our animal model. Our previous study showing reduction of injury by treatment with dexamethasone indicates that the effect of therapies on injury can be reliably studied in the model [4].

Excitatory amino acids (EAA) are increasingly implicated in the pathogenesis of neuronal injury induced by a variety of central nervous system insults, such as ischemia and trauma. An amplifying effect on central nervous system injury has been linked to the stimulation of the neuronal N-methyl-D-asparate (NMDA) subtype of EAA receptors by glutamate, the most abundant of the EAA neurotransmitters. We have previously documented an increase of glutamate concentrations in brain interstitial and cerebrospinal fluid (CSF) during pneumococcal meningitis in rabbits [6]. Comparable results were obtained in a similar model of Escherichia coli meningitis [7]. This suggests that EAA may contribute to neuronal injury in bacterial meningitis.

In the present study, we further examined the role of EAA in causing neuronal injury during meningitis by examining the effect of EAA inhibition on neuronal injury in our rat model of neonatal meningitis. Kynurenic acid, a nonselective EAA antagonist active at all EAA receptor subtypes, including the NMDA receptor, has been shown to reverse glutamate neurotoxicity in cell cultures and to exert neuroprotective effects in animal models of ischemic and traumatic brain injury, including models of neonatal brain injury [8–10]. We chose kynurenic acid for the present studies because of its lack of significant toxicity in infant rats and its potent neuroprotective effect.

Materials and Methods

Infecting organism. A strain of group B streptococci type III, one of the most common types causing neonatal meningitis (provided by Craig Rubens, University of Washington, Seattle), was used as described [4]. The organism was cultured overnight in Todd-Hewitt broth, then diluted in fresh medium, grown to logarithmic phase, pelleted, and resuspended in normal saline to the desired density and used to infect the infant rats.

Model of meningitis. Nursing Sprague-Dawley rat pups with their dam were purchased (Simonsen, Gilroy, CA) and infected...
on postnatal day 11–14 by direct intracisternal injection of 10 μL of a suspension of the infecting organism, using a 32-gauge needle. The inoculum size varied between $5 \times 10^4$ and $1 \times 10^6$ cfu/mL. Randomized treatment with kynurenic acid (300 mg/kg subcutaneously once daily; $n = 20$; Sigma, St. Louis) or saline ($n = 17$) was started at the time of infection. Eighteen hours later, pups were assessed clinically for the presence of seizures and their ability to right themselves and ambulate. A small sample of CSF (10 μL) was obtained by puncture of the cisterna magna and was cultured quantitatively as described below to document meningitis. Animals were then treated with antibiotics (ceftriaxone, 100 mg/kg subcutaneously once daily; Hoffmann-La Roche, Nutley, NJ). Animals were euthanatized on day 1, 2, or 3 after infection by intraperitoneal injection of sodium pentobarbital (200 mg/kg), and brains were processed for histopathology as described below.

**Bacterial cultures.** Serial dilutions of CSF in saline were cultured on blood agar plates incubated for 24 h at 37°C in room air with 5% CO₂.

**Brain preparation.** Immediately after euthanasia, animals were perfused via the left cardiac ventricle with 60 mL of 4% paraformaldehyde in PBS (pH 7.4). Brains were removed and postfixed overnight in the same fixative solution at 4°C, then placed in 30% phosphate-buffered sucrose and cut at 40- to 60-μm intervals on a vibratome [11]. Sections were mounted on gelatinized glass slides for staining. After dehydration, sections were Nissl-stained in cresyl violet, and slides were rehydrated and coverslips were fixed with Permount [11].

**End point analysis.** Clinical severity of disease was scored in each animal at 18 h of infection and was graded as follows: 4 = normal activity and ambulation; 3 = reduced ambulation; 2 = slow righting; 1 = unable to right within 5 s. At the same time, the presence or absence of tonic seizures was recorded. Brain sections stained with cresyl violet (Nissl) were examined for the presence of inflammation in the subarachnoid space and ventricles, which was scored semiquantitatively (0 = no inflammation; 1 = occasional inflammatory cells; 2 = inflammatory cells forming an infiltrate not involving the entire depth of the subarachnoid space; 3 = inflammatory infiltrate in the entire subarachnoid space). Cortical neuronal injury was defined as areas of decreased density of neurons or frank cortical necrosis and was quantitated. Twelve cortical sections spanning the middle half of the brain were scored in each animal for the presence or absence of neuronal injury (figure 1), and the average injury score for each individual animal was used for statistical analysis. Similarly, injury to the dentate granule cell layer of the hippocampus was scored in each section containing the structure (see figure 1). Histopathologic evaluations were done by an investigator (M.G.T.) blinded to the clinical, microbiologic, and treatment data of animals.

**Statistics.** Bacterial titers are presented as mean ± SD, and differences between groups were analyzed by Student's $t$ tests. Proportions between groups were compared by Fisher's exact test. Scores between groups were compared by Kruskal-Wallis test. The correlation between scores was tested by Spearman's rank-order correlation.

**Results**

**Clinical disease.** By 18 h after infection, meningitis was fully developed in all infected animals. The majority of infected animals were lethargic to obtunded, and our clinical scoring system did not detect significant differences between animals treated with kynurenic acid and control animals (in both groups, median score, 2; range, 1–3; not significant). During an observation period of 2 h, there was also no significant difference in seizure incidence between groups (18% in controls vs. 10% in treated animals; not significant). CSF bacterial titers were also almost identical in the treated and control groups (log₁₀ $8.9 ± 1.2$ vs. $9.1 ± 0.6$ cfu/mL; not significant).

**Histopathology.** The brain histopathology in these experiments was characterized by subarachnoid and ventricular granulocytic inflammation, neuronal injury, and cortical infarcts. Intense granulocytic inflammation involving the subarachnoid and ventricular space was present in all infected animals, without significant difference in score between experimental groups (in both groups, median, 3; range, 2–3; not significant). Uninfected animals treated with kynurenic acid showed neither inflammation nor neuronal injury.

Nissl staining of neurons with cresyl violet revealed two major types of neuronal injury: cortical neuronal necrosis (figure 2A–F) and injury to the dentate granule neurons of the hippocampus (figure 2G). Duration of survival after induction of infection (24–72 h) had no detectable influence on the score for both types of injury, and at each time point, injury was more severe in controls than in treated animals (figure 2). The morphology of injury was relatively diffuse 1 day after infec-
tion (figure 2A, B), while injured areas became more clearly demarcated in animals surviving for 2 or 3 days (figure 2C–F). In the cortex, neuronal injury ranged from occasional foci of reduced cell density (figure 2D) to large areas of complete necrosis (figure 2C, E, F), the latter frequently associated with hemorrhage. In control animals, the extent of injury ranged from a score of 0 (no injury) to 6.8, with a median of 1.25. In contrast, animals treated with kynurenic acid had a score ranging from 0 to 2.58 (median, 0). The difference between infected animals treated with kynurenic acid and infected controls was significant ($P = .027$; figure 3A).

The hippocampal injury in this model was almost completely restricted to the dentate granule cell layer (figure 2G), with the lower blade being more commonly affected than the upper blade (in 45 individual sections showing hippocampal injury, involvement of the lower blades outnumbered that of upper blades by 44 to 9; $P < .001$). While 7 of 17 control animals had hippocampal injury, only 2 of 20 in the kynurenate group had injury (figure 3B; $P = .029$). Cortical injury and hippocampal injury scores of all studied animals showed a close positive correlation ($r = .75$, $P < .001$).

**Discussion**

The results of the present study demonstrate a beneficial effect of kynurenic acid on the extent of neuronal injury in a rat model of neonatal meningitis caused by GBS. Specifically, the extent of neuronal cell loss in the hemispheric cortex and in the hippocampal dentate granule cell layers was markedly reduced. To our knowledge, this is the first report to demonstrate a neuroprotective effect of an EAA antagonist in an animal model of meningitis.

EAA receptors are classified into several subtypes by their preferential agonists [12]. NMDA receptors, termed after their most potent agonist, N-methyl-D-aspartate, are receptor-gated ion channels that have been implicated in long-term potentiation and in mediating excitotoxic neuronal injury, the latter particularly in the developing brain [13, 14]. NMDA receptor activation by glutamate leads to influx of calcium into the cell and depends on binding of glycine to a site on the receptor. Kynurenic acid binds noncompetitively to the glycine site of

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effect of kynurenic acid (300 mg/kg subcutaneously once daily) on neuronal injury scores in cortex (A) and hippocampus (B) in infant rats with group B streptococcal meningitis. Control animals received equal volumes of saline. Injury was scored 24–72 h after infection.
the NMDA receptor, thus inhibiting receptor activation and calcium flux [15]. Activity at non-NMDA receptor types (AMPA-kainate) may also contribute to the neuroprotective effect of kynurenic acid [15].

Previous studies have found that concentrations of EAA in the CSF increase substantially during meningitis [16]. Under physiologic conditions, the concentration of glutamate in the CSF is <25% of that found in plasma [17]. Active uptake of EAA from the CSF by the choroid plexus and restricted entry through the blood-brain barrier maintain a low glutamate level in CSF [17]. Two early and prominent pathophysiologic changes in meningitis, disruption of the blood-brain barrier and choroid plexitis, could account for a sustained increase in CSF glutamate concentration [6].

Brain structures adjacent to the ventricles are freely exposed to the high CSF glutamate concentrations, as the ependymal cell lining of the ventricle is highly permeable to diffusion [17]. It is conceivable that the high glutamate concentrations in the CSF contribute to some of the neuronal toxicity observed in our model. The lower blade of the dentate granule layer, which is anatomically closest to the ventricle, was more commonly affected than the more distant upper blade. This preferential vulnerability may reflect decreasing concentrations of glutamate in brain interstitial fluid with increasing distance from the ventricular CSF. The possibility of a local factor responsible for the hippocampal injury is supported by the fact that the dentate granule cells, in contrast to other structures in the hippocampus, are not very sensitive to ischemic injury [18], yet they were the only hippocampal structure regularly injured in the meningitis model. Mediators other than EAA present in the ventricular CSF, which uniformly shows a dense granulocytic inflammation in our model, may also contribute to the dentate granule cell injury.

In addition to the marked increase in EAA in the CSF during meningitis, we and others have previously shown that glutamate concentrations in the brain interstitial fluid are significantly increased in experimental meningitis [6, 7]. The extracellular concentration of glutamate in the brain is tightly controlled under normal circumstances by astrocytes, which take up and metabolize glutamate. Such tight control of interstitial glutamate concentrations appears critical for neuronal survival [19, 20]. When the brain is subjected to a variety of insults, including trauma and ischemia, increased release and decreased uptake of glutamate in injured neurons and glia may cause the interstitial fluid concentration to rise to neurotoxic levels and propagate further neuronal injury.

Ischemia is likely to be responsible for the release of EAA and thus some of the cortical neuronal injury in our model. Inflammation in the subarachnoid space surrounds blood vessels and extends into the cortical parenchyma along the Virchow-Robins spaces. The resulting vasculitis is a common feature of meningitis [21], causing temporary vasospasm, which may lead to thrombosis and vascular occlusion [22, 23]. The pathophysiologic consequences of bacterial meningitis, including brain edema, increased intracranial pressure, systemic hypotension, and impaired cerebral blood flow autoregulation, can lead to a global decrease of cerebral blood flow [24]. In clinical studies of advanced meningitis [22, 23] and in experimental models [25, 26], cerebral blood flow has been found to be reduced. These vascular changes may result in local ischemic damage with subsequent glutamate release and development of excitotoxic injury. The beneficial effect of kynurenic acid in this model is likely related to the protection from excitotoxic neuronal injury through inhibition of EAA receptors. In addition, a reduction of brain edema resulting from EAA inhibition [9] may have more indirect beneficial effects by reducing intracranial pressure and improving cerebral blood flow.

Kynurenic acid has anticonvulsive activity [15], but it is unclear whether this property is of importance for the neuroprotective effect of the drug in our model. In the immature rat brain, seizures, including status epilepticus, have not been clearly linked to the development of neuronal injury [27]. Furthermore, the monitoring for seizures during a limited observation period showed only an insignificant reduction of seizure frequency in the treated group.

Further studies will be needed to define the time window of effectiveness of EAA antagonists in the treatment of bacterial meningitis and to explore the relative potency and toxicity of antagonists with different modes of action. Nevertheless, the data presented in this study confirm the previously proposed concept that EAA may contribute to neuronal injury in bacterial meningitis and indicate that this class of neurotoxic molecules may represent an important target for adjunctive therapy for bacterial meningitis.

Acknowledgments

We thank R. Ann Sheldon, QingXiang Liu, and Berkley R. Elliot, Jr., for excellent technical support.

References