論文の内容の要旨

獣医学　専攻

平成 14 年度博士課程　進学

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論文題目

Studies on the pathophysiological states of canine necrotizing meningoencephalitis

（犬の壊死性髄膜脳炎における病態生理に関する研究）

Canine necrotizing meningoencephalitis (NME) is an unique form of non-suppurative inflammatory disease, often called "Pug dog encephalitis”. The common clinical signs of dogs with NME are forebrain signs, such as partial or generalized seizure, decreased consciousness, abnormal behavior, circling, and ataxia. Histopathologically, canine NME is characterized by a lymphocytic-plasmacytic inflammation, chronic neurodegeneration with necrotic foci, and activated astrocytes around lesions. Although the pathogenesis of NME remains unclear, previous studies suggested that NME is an autoimmune disease, detected an anti-astrocyte autoantibody in

cerebrospinal fluids (CSFs). On the other hand, many researchers demonstrated that an excess of excitatory amino acids, such as glutamate and aspartate, play an important roles in the neuronal cell degeneration and/or cell death via the synaptic excitatory amino acid receptors, including neural N-methyl-D-aspartate (NMDA) receptor. Excitatory amino acids increase cytoplasmic free Ca+2 concentration and induce subsequent neuronal cell death.

In the present study, based on a hypothesis that the parenchymal necrosis in the NME is caused by the glutamate toxicity, excitatory amino acids (glutamate and aspartate) in CSFs were measured in dogs with NME in chapter 1. In chapter 2, the effect of NME-CSF on glutamate transport of astrocytes was examined using *in vitro* model. The effect of glutamate transporter inhibitor was also examined. In addition, expressions of glutamate transporters (GLT-1 and GLAST) mRNA were investigated.

Chapter 1: Evaluation of excitatory and inhibitory amino acids in CSF of dogs with NME

Total 34 dogs (6-month to 7-year of age, 11 males, 14 females, 6 neutered-females, and 3 neutered-males), all of which were diagnosed as NME by magnetic resonance imaging (MRI) and/or postmortem pathological examinations, were used. Samples of CSF were collected from the cisterna magna under the sedation. All samples were positive for anti-astrocyte autoantibody. Ten CSF samples from clinically healthy Beagle dogs were used as the control.

Concentrations of exicitatory (glutamate and aspartate) and inhibitory amino acids (taurine and γ-aminobutyric acid: GABA) in CSFs were measured by a high performance liquid chromatographic (HPLC) method with some modifications. In NME

cases, glutamate concentrations (8.16 ± 7.8 μM: mean ± SD) in CSFs were significantly higher than those in healthy controls (1.40 ± 0.8 μM: $p<0.001$). Significantly higher aspartate concentrations (0.87 ± 0.6 μM) were also found in NME-CSFs in comparison with healthy controls (0.21 ± 0.3 μM: $p<0.01$). While, taurine concentrations were also significantly higher in NME-CSFs (2.79 ± 1.7 μM) than those in controls (1.81 ± 0.6 μM: $p<0.01$). No significant difference in GABA concentrations in CSFs was observed between NME cases and healthy controls. These results indicated that extracellular glutamate, aspartate, and taurine concentrations increased in the brain of NME dogs. The increase of extracellular glutamate, produced by excessive release and/or inadequate uptake by astrocytes, may induce an overstimulation of glutamate receptors on neurons and result in neuronal cell swelling and lysis. Therefore, glutamate-induced excitotoxicity was considered to be one of possible causes for progressive neurodegeneration observed in dogs with NME.

Chapter 2. Effects of NME-CSF on glutamate transport in astrocytes

To clarify the mechanism of elevated glutamate concentration in CSF observed in NME dogs, confluent canine astrocytes were cultured with NME-CSFs as an *in vitro* model. The increments of supernatant glutamate, aspartate, and taurine concentrations after the cultivation with NME-CSFs were significant higher (20.9 ± 8.6, 1.04 ± 0.6, 2.90 ± 1.3 μM, respectively) than those with control CSFs (0.06 ± 0.02, 0.08 ± 0.06, 0 ± 0.01 μM, respectivery: $p<0.01$). Therefore, NME-CSFs induced increase of glutamate release and/or decrease of glutamate uptake in astrocytes. In addition, when the astrocytes were pre-incubated with a glutamate transporter

inhibitor (L-trans-2,4-PDC), supernatant glutamate concentration was significantly decreased from 10.98 μM to 6.27 μ M ($p<0.05$). Hence the increase of glutamate concentration in NME-CSFs was closely related to glutamate transporters, through of which remove extracellular glutamate. The NME-CSFs were considered to reduce glutamate uptake by astrocytes, resulting in an excess of extracellular glutamate concentration. In addition, expression of GLT-1 and GLAST mRNA, especially GLT-1 mRNA was decreased in astrocytes after the cultivation with NME-CSF, also suggesting the decrease of glutamate transport in aastrocytes.

In conclusion, glutamate excitotoxicity is closely related to induce the progressive neurodegeneration and neuronal cell death in dogs with NME. Certain factor in CSF of NME dogs induced an excessive extracelllular glutamate concentration by the decrease of glutamate uptake in astrocytes.