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Clinical and experimental studies of epilepsy associated with focal cortical dysplasia

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Running title: Clinical and basic studies on focal cortical dysplasia

Abstract

The results of clinical and experimental studies on epilepsy associated with focal cortical dysplasia (FCD) are presented.

We have been interested in findings of abnormal increases in numbers of small vessels in specimens of FCD resected from epilepsy patients. In the clinical study, specimens of FCD or dysembryoplastic neuroepithelial tumor (DNT) from 13 patients of epilepsy were examined by immunohistochemistry. The numbers of vessels in both lesions were greater than those in cortical specimens of autopsy cases without epilepsy. Because the vessels showed negative staining of VEGF, it was thought that the phenomenon of increase in number of vessels was simply a hypervascularity, not a neovascularity. The local hypervascularity was expected to show local hyperperfusion in CBF-SPECT study, but interictal SPECT demonstrated local hypoperfusion and ictal SPECT showed hyperperfusin. This may have been caused by a functional change in those vessels.

In the experimental study, we tried to make a new animal model of FCD to study epileptogenisity of FCD. When kainic acid had been infused into the neocortex in the neonatal rats, FCD was induced in adult Wistar rats. Histopathological examination revealed cortical dyslamination and abnormal neurons. On EEG, local spike bursts were elicited from the lesions. However, clinical seizures were not detected. Although the data are preliminary and observation over a longer period is required to determine whether spontaneous seizures will occur in this model, it is expected that this new model will be useful for studying epilepsy associated with FCD.

Key words:
focal cortical dysplasia, epilepsy, histopathology, capillary vessels, animal model

Introduction

High-resolution MR imaging has made it possible to detect small neocortical lesions in some epilepsy patients that could not be detected previously[7,14]. Recently, focal cortical dysplasia (FCD) has become recognized as a surgically curable pathogenesis of refractory epilepsy[13]. Histopathologically, FCD not only shows cortical dysgenesis and/or abnormal neurons but is also accompanied by abnormal white matter under the cortical lesions [5].

The process of construction of the cerebral cortex starts from proliferation of neuroblasts in the germinal matrix layer around the ventricle of fetal age of 6-7 weeks and progresses to neuronal migration to the cerebral surface and organization of the cortical layers. Even in human, this process is not completed at birth; it continues for 2-3 days after birth. Thus, prevention of this process, even in the postnatal period, results in the development of cortical dysplasia [2,3,16]. Since other elements of the brain are also developing during this process, the prevention of this process may result in dysgenesis of not only neurons but also other elements such as glial cells.

We have been interested in histopathological findings of abnormal increases in numbers of small vessels in specimens of FCD resected from epilepsy patients. In this study, we examined the small vessels of FCD by immunohistochemistry and compared the findings with CBF-SPECT findings.

The epileptogenic zone in patients with FCD is thought to exist in the lesion itself, but there is also a possibility that it exists in the neighboring cortex of the lesion as is seen in brain tumors. Studies on epileptogenisity of FCD using appropriate animal models are required. We tried to establish a new animal model of FCD by a postnatal procedure, and the preliminary data were presented.

Increase in number of vessels of FCD and SPECT findings

Materials and Methods

We have experienced consecutive surgical series of seven FCD and six dysembryoplastic neuroepithelial tumor (DNT) patients with epilepsy in 1990-1999. The surgical specimens were stained with H-E, CD31, VEGF and MIB-1. CD31 stains the endothelium of vessels, VEGF is a marker of neovascularization, and MIB-1 index is a marker of tissue proliferation. The number of vessels was counted by a coauthor, Dr. Hodozuka, in a slice of CD31 staining of high power field (x 200), and the average of the numbers in 10 fields was used for analysis. The number of vessels, percentage of vessels for positive VEGF, and MIB-1 index of the lesion were analyzed in the FCD, DNT and control groups. The control consisted of microscopically normal-appearing cortical specimens from 11 autopsy cases with no history of epilepsy. The data were statistically analyzed by Mann-Whitney U test. The histopathological findings were compared with the findings of ^{99m}Tc-ECD SPECT in the patients. Interictal SPECT was performed in all epilepsy patients, and ictal SPECT was performed in five of the FCD and three of the DNT patients. Ictal SPECT images were obtained by the tracer injection during or immediately after seizures.

Results

An increase in the number of small vessels was observed six of the seven FCD and five of the six DNT patients (Figure 1). The average numbers of vessels in FCD and DNT were significantly greater than that in the control (Table 1). There was no difference between the numbers of vessels in FCD and DNT. None of the specimens were stained by either VEGF or MIB-1 in FCD, DNT and the control. In interictal SPECT, five of the FCD and five of the DNT patients showed local hypoprefusion areas, and the remained patients showed no abnormal perfusion areas. In ictal SPECT, five of the FCD and one of the DNT patients showed local hyperperfusion, and two of

the DNT patients showed local hypoperfusion.

Discussions

Although neither FCD nor DNT is a growing lesion, as was shown by the negative results for MIB-1 index, the numbers of vessels in both lesions were greater than that in the control. The negative results for VEGF indicate that the increase in the number of vessels is simply a hypervascularization, not a neovascularization. The results suggested that the increase in the number of vessels in FCD and DNT was a malformative change, including neuronal and glial dysgenesis. The local hypervascularity was expected to show local hyperperfusion in CBF-SPECT study, but the lesions showed no hyperperfusion in interictal SPECT. In contrast, ictal SPECT demonstrated a local hyperperfusion area similarly to the previous reports[9]. These are interesting findings, and it is difficult to explain the discrepancy between histological findings and CBF changes, but it may be caused by a functional change in vessels according to tissue demand. Further studies of hypervascularity in epileptic lesions are needed in larger series.

A new animal model of FCD

Materials and Methods

We tried to make a new animal model of FCD by postnatal treatment with kainic acid (KA), which is an excitatory amino acid. Wistar rats of postnatal age 0-1 days were restrained in a half plaster cast under light halothane anesthesia. The skin and skull were perforated near the unilateral sensorimotor cortex (just anterior to the bregma and 2 mm lateral to the midline) using a 24-gauge injection needle. A stainless-steel cannula, 0.3 mm in outer diameter, was inserted into the cerebral cortex (1.5 mm deep from the skin), and 0.5 μl of KA solution of was infused slowly. The concentrations of KA solution used were 0.5, 1.0 and 2.0 μg/μl. Since it is important to maintain body temperature during the procedure, the neonatal rats were kept together with their mothers. At 4, 8 and 12 weeks after the procedure, the animals were sacrificed and examined histopathologically. In the animals aged 12 weeks or more, screw electrodes were stereotacticaly implanted in the skull for EEG recording. Behavior and EEG were continuously recorded for eight hours using a video-EEG system.

Results

The neonatal rats tolerated the procedure and continued to grow. Histopathologically, cortical dyslamination was found around the region of KA infusion in the animals aged 4 weeks or more. Abnormal neurons were also observed there, which showed abnormal polarity and/or pyknosis (Figure 2). EEG showed frequent local spike bursts in the KA-treated region, but clinical seizures were not observed (Figure 3).

Discussions

Previously reported animal models of FCD can be classified into three types: genetic, fetal insult and neonatal lesion models[4]. TISH mutant rats, Reeler mutant rats, Ihara mutant rats and p53-knockout mice were well

known as genetic models of FCD. As fetal insult models, fetal irradiation and intrauterine administration of methylazoxymethanol acetate have been used to induce FCD[8]. A neonatal freeze lesion model has demonstrated a clear four-layered cortex with a microgyrus[6], and a similar effect was obtained by a cortical injection of ibotenic acid in neonatal rats. To study epileptogenesis of FCD, an animal model is useful that shows a uniformly localized lesion and spontaneous seizures. However, in the previous models, spontaneous seizures were observed only in genetic models that showed bilateral or diffuse lesions. Fetal insult models showed only electrographic seizure discharges [1], and those demonstrated various cerebral malformations. Freeze lesion and intracortical ibotenate models demonstrated localized lesions but no seizures[10,15]. We infused KA into the cortex of the neonatal rat to make a new model of FCD that shows spontaneous seizures. Intracortical infusion of KA induced cortical dyslamination and generation of abnormal neurons. These findings are compatible with FCD of Palmini's grade 2 [11], although EEG showed only epileptic discharges, which did not develop into clinical seizures. Observations over a longer period are needed to determine whether spontaneous seizures will occur in this model. Although the data obtained from animal experiments can not be directly applied to human epilepsy, it is expected that this new model will be useful for studying epilepsy associated with FCD.

Conclusions

We presented an interesting histopathological finding and a new experimental model of FCD. We hope that these studies will contribute to clarification of the epileptogenic mechanism of FCD and to the development of effective treatment for these epilepsy patients.

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Figure Legends

Fig. 1:
Photomicrographs of focal cortical dysplasia from an epilepsy patient
A: H-E x 40, B: H-E x 100. Dyslamination of the cortex is seen in a gyrus, and disorganization of white matter is also noted. Increase in vessels is demonstrated there. C: VEGF x 200, D: MIB-1 x 200. The vessels are not stained with VEGF and MIB-1. Scale bar = 200 μm

Fig. 2:
Photomicrographs of focal cortical dysplasia induced by kainic acid in rats
Left: Cresyl-violet stain x 40. A pseudosulcus is noted in the cortical surface, and focal cortical dysplasia is induced around the abnormal sulcus.
Right: Cresyl-violet stain x 200. There are abnormal neurons, which shows multipolarity and pyknosis. Scale bar = 200 μm.

Fig. 3:
Electroencephalogram in the animal model of focal cortical dysplasia
Spike burst is frequently recorded in RSMC-f that kainic acid was injected.
LSMC, RSMC: left or right sensorimotor cortex. –f: forelimb area, -h: hindlimb area.

Fig. 1

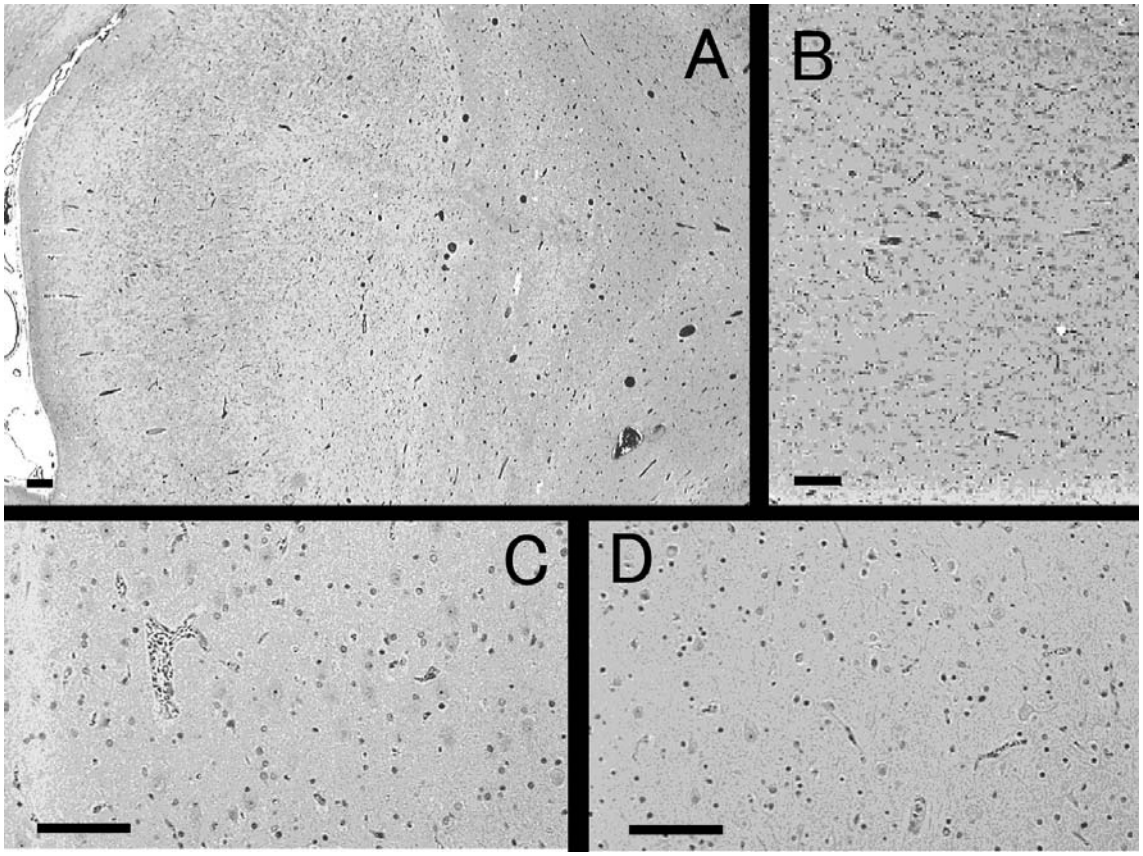

Fig. 2

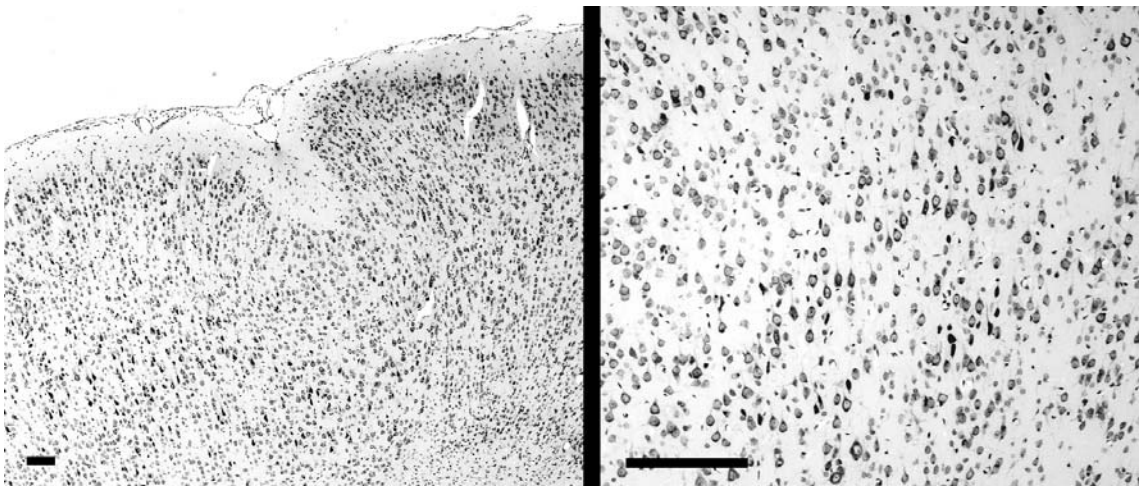

Fig. 3