Epileptogenic Properties of Balloon Cells in Cortical Tubers of Tuberous Sclerosis: Upregulation of Drug Resistance Proteins

Nam-Gu Kang, M.D.,¹ Hong-Joon Chang, M.D.,¹ Young-Cheol Ok, M.D.,¹ Rae-Seop Lee, M.D.,¹ Seung-Kyu Park, M.D.,¹ Jun-Seob Lim, M.D.,¹ Kyu-Yong Cho, M.D.,¹ Hyung-Ihl Kim, M.D.,² Jae-Hyoo Kim, M.D.,³ Hyun-Sik Oh, M.D.,³ Min-Cheol Lee, M.D.⁴

Department of Neurosurgery,¹ Gwangju Christian Hospital, Gwangju, Korea
Department of Neurosurgery,² Jeonju Presbyterian Hospital, Jeonju, Korea
Departments of Neurosurgery,³ Pathology,⁴ Chonnam National University Medical School, Gwangju, Korea

Objective: Balloon cells and dysplastic neurons are histopathological hallmarks of the cortical tubers of tuberous sclerosis complex (TSC) and focal cortical dysplasia (FCD) of the Taylor type. They are believed to be the epileptogenic substrate and cause therapeutic drug resistant epilepsy in man. P-glycoprotein (P-gp) is the product of multidrug resistance gene (MDR1), and it maintains intracellular drug concentration at a relatively low level. The authors investigated expression of P-gp in balloon cells and dysplastic neurons of cortical tubers in patients with TSC.

Methods: An immunohistochemical study using the primary antibody for P-gp, as an indicative of drug resistance, was performed in the cortical tuber tissues in two patients of surgical resection for epilepsy and six autopsy cases.

Results: Balloon cells of each lesion showed different intensity and number in P-gp immunopositivity. P-gp immunopositivity in balloon cells was 28.2%, and dysplastic neurons were 22.7%. These immunoreactivities were more prominent in balloon cells distributed in the subpial region than deeper region of the cortical tubers. Capillary endothelial cells within the cortical tubers also showed P-gp immunopositivity.

Conclusion: In this study, the drug resistance protein P-glycoprotein in balloon cells and dysplastic neurons might explain medically refractory epilepsy in TSC.

KEY WORDS: Balloon cells · Dysplastic neurons · Multidrug resistance · P-glycoprotein · Tuberous sclerosis complex (TSC).

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem disorder which is characterized by mental retardation, epilepsy, and tumors of skin, retina, heart, kidney, and brain.²⁴ The systemic lesions are caused by mutations in tumor suppressor genes TSC1 (chromosome 9q34) or TSC2 (chromosome 16p13.3), which encode the proteins hamartin and tuberin, respectively.⁵,²⁰ TSC lesions show abnormalities in cell proliferation, differentiation, and migration suggesting a role for the encoding protein in these cellular processes. There is an evidence to suggest that the TSC2 product, tuberin, suppresses tumorigenicity.⁴,²³ The tumors developed in patients with TSC are mainly benign, including subependymal giant cell astrocytomas (SEGA), facial angiofibromas, renal angiomyolipomas, and cardiac rhabdomyomas,⁷,³⁷ and they are associated with loss of heterozygosity for TSC1 or TSC2 genes. These cytogenetic abnormalities support the tumor suppressor gene hypothesis in the tumorigenesis of TSC.

In the central nervous system (CNS), patients with TSC develop cortical tubers and/or SEGA.³⁷ Cortical tuber is a localized malformation of the neocortex and underlying white matter, which belong to categories of malformations of cerebral cortical development (MCD) or neuronal migration disorders (NMDs). NMDs result from disturbed organogenesis and involve cells that form the cerebral cortex,¹²,²¹ and they have
become an important aspect of medically intractable epilepsy. The histopathologic features of cortical tuber include cortical dyslamination of variable severity, presence of glioneuronal cell nests in the cortical layer I, heterotopic neurons in the white matter, and dysmorphic neurons and balloon cells.\(^1\)

Moreover, the presence of balloon cells is the histopathologic hallmark of Taylor's type of focal cortical dysplasia (ECD), equivalent to grade IV of NMD, and cortical tubers in TSC\(^1\).

\(\text{P-}\)glycoprotein (P-gp), "P" meaning permeability, is an ATP-dependent drug transport protein, and the original investigators speculated that P-gp decreases the fluidity of hydrophobic domains of membranes and restricts access of drugs to the cytoplasm of cancer cells.\(^2\) In the subsequent studies, P-gp is the product of multidrug resistance gene (MDR1) located to chromosome 7q21, and acts as an ATP-dependent membrane efflux transporter instead of the regulation of membrane permeability.\(^3\) It maintains intracellular drug concentration at a relatively low level, so called a multidrug resistant protein. Multidrug resistance for many chemotherapeutic agents including doxorubicin and vincristine in cancers and brain tumors has been found to be due to the overexpression of MDR1 and P-gp.\(^4\)\(^5\)\(^6\)\(^7\)

It is estimated that 20-25% of epileptic patients are not likely to relieve seizures treated with antiepileptic drugs (AED).\(^8\)\(^9\) Recently, increased P-gp expression in blood-brain barrier (BBB) has been described in epileptogenic brain tissue of patients with medically intractable epilepsy, suggesting that overexpression of P-gp may be involved multidrug resistance of epilepsy.\(^10\) In the present study, we investigated expression of P-gp in balloon cells and dysplastic neurons of the epileptogenic brains in patients with TSC surgically treated for their pharmaco-resistant epilepsy and cortical tubers of TSC confirmed by postmortem autopsy studies.

**Materials and Methods**

**Case selection and tissue section preparation**

Eight cases consisting of two patients and six autopsy cases were selected (Table 1). Two patients were compatible with diagnostic criteria of TSC with medically intractable epilepsy and undertaken epilepsy surgery. Resected frontal and temporal neocortical tissues were consistent with primary epileptogenic substrates as evaluated by clinical seizure patterns, and MR images associated with surface and invasive electroencephalography. Six autopsy cases also had clinical history of epilepsy in their medical records. The tissues used for pathological evaluations were serially dissected to 3 mm and examined grossly and histologically. Gross architectural abnormalities, if present, are described. The presence of coexistent heterotopia within the white matter, subpial gliosis, and presurgical evaluations of

**Table 1. Clinicopathologic features of 8 cases with tuberous sclerosis complex and drug-resistant epilepsy**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age</th>
<th>Sex</th>
<th>Epileptic seizures</th>
<th>Associated lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>F</td>
<td>CPS</td>
<td>RC, HM</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>M</td>
<td>CPS</td>
<td>AF, HM</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>M</td>
<td>IS, CPS</td>
<td>CR</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>F</td>
<td>GTC, CPS</td>
<td>SEGA, HM</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>M</td>
<td>CPS, MS</td>
<td>AS</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>M</td>
<td>GTC, CPS</td>
<td>AF, SEGA</td>
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<tr>
<td>7</td>
<td>47</td>
<td>M</td>
<td>CPS</td>
<td>RC, HM</td>
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<tr>
<td>8</td>
<td>38</td>
<td>F</td>
<td>CPS</td>
<td>AS, HM</td>
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epileptogenic area related lesions (contusions and infarctions) were described.

Tissue sections were prepared from formalin-fixed paraffinembedded tissues. The sections used were 6 μm thick and were stained with hematoxylin and eosin (H&E). The number of tissue sections examined per case ranged from 7 to 21 (mean, 13 sections). In six autopsy cases, paraffin-embedded tissue blocks obtained from cortical tuber were used. For histopathologic diagnosis of TSC from the surgical and autopsy brains, additional special staining, such as, Bielschowsky's silver staining, and immunohistochemical staining for glial fibrillary acidic protein (GFAP), medium and high molecular weight neurofilament protein (NF-M/H), and vimentin were routinely performed. Two representative tissue sections bearing many balloon cells in each TSC lesion were used in this study.

**Immunohistochemistry**

To detect expression of P-gp in cortical tuber of TSC patients with drug-resistance to epilepsy, immunohistochemistry for monoclonal MDR-1 antibody (1:100, Clone JSB-1, Novocastra, Newcastle upon Tyne, UK) was performed. Tissue sections were collected on probe-glass slides (Fisher Scientific, Pittsburgh, PA, USA), and immunostained using the avidin-biotin conjugation method\(^11\). Endogenous peroxidase activity was blocked with incubating sections in phosphate buffered saline (PBS, pH7.4) containing 1.5% H.O. The slides were then treated with 10% normal serum in PBS for 30 min in order to block charged sites on the tissue surface, and then with each primary antibody overnight at 4°C. A streptavidin-horseradish peroxidase (Research Genetics, Huntsville, AL, USA) detection system was then applied. After treatment with 1% avidin-biotin horseradish peroxidase complex for 1 hr at room temperature, the immunopositivity of tissue sections were visualized by chromogen reactions with 3-amino-9 ethyl carbazole (AEC, Biomega, Foster City, CA, USA). Sections then were
counterstained with hematoxylin, and examined under a light microscope. The frontal and temporal cortex obtained from adult autopsy brains without any neurologic disease served as disease-free controls for primary antibodies. Primary antibody was substituted for PBS as a negative control.

**Results**

Clinicopathologic features of cortical tuber in TSC

In two patients who underwent surgical resection for epilepsy, one patient had a solitary cortical tuber located in the frontal cortex, and the other patient had multiple cortical tubers in the frontal and temporal cortex (Fig. 1). In six autopsy cases, four cases showed one cortical tuber in the frontal cortex, and two of them were associated with SEGA. Other two autopsy cases showed multiple cortical tubers in the frontal and parietal cortex. The cut surface of cortical tuber revealed a well-defined, nodular, whitish sclerotic lesion involved in the gray and subcortical white matter. The gray and white matter junction of the lesion was blurred, while adjacent normal cortex showed clearly defined gray and white matter. An old infarct healed by brownish cystic gliosis was present in the vicinity of cortical tuber in one patient (Fig. 2A). Lesions demonstrated the characteristic histopathologic findings consisting of loss of laminar arrangements of neurons, presence of enlarged dysplastic neurons, and balloon cells. Balloon cells in TSC were large, round cells with oval, eccentric nuclei, prominent nucleoli and abundant eosinophilic cytoplasm with a few enlarged processes (Fig. 2B). They usually presented in scattered cells throughout the gray matter, and rarely in clusters. Balloon cells with dysplastic neurons were observed in the white matter in one TSC patient. Immunohistochemical analysis of balloon cells yielded variable immunopositivity (Fig. 2C, D) for GFAP (56.7%), NF-M/H (35.2%), and vimentin (67.3%).

In control temporal cortex, P-gp immunopositivity was expressed in capillary endothelial cells, but no P-gp immunopositivity was noted in neurons or astrocytes (Fig. 3A). However, the cortical tubers containing balloon cells, enlarged dysplastic neurons and fibrillary astrocytes showed P-gp immunopositivity in all eight cases (Fig. 3B, C, D). Balloon cells of each lesion showed different intensity and number in P-gp immunopositivity. P-gp immunopositivity in balloon cells were 28.2%, and dysplastic neurons were 22.7%. These immunoreactivities were more prominent in balloon cells distributed in the subpial region than deeper region of the cortical tubers. Capillary endothelial cells within the cortical tubers also showed P-gp immunopositivity.

**Discussion**

In the present study, variable proportions of balloon cells in TSC showed immunoposivities for GFAP, NF-M/H, and vimentin indicating that there were different subclones of balloon cells, namely, glial, neuronal, and mixed. These immunohistochemical characteristics suggest that balloon cells are derived from the incomplete
maturation of neuroepithelial stem cells during early stage of cortical development\textsuperscript{4,10}. These cells appear as randomly scattered or clustered cells in a disorganized cortex.

Long term treatment of any single or combination of AEDs is the standard therapeutic approach to control epileptic seizures, however, about 20-25\% of patients do not show good response to AED treatment\textsuperscript{3,29}. Intrinsic resistance of AEDs may be caused by three main mechanisms; the first is loss of pharmacological target due to neuronal cell death, the second is poor penetration of drug into the epileptogenic substrates, and third is intracellular transport and drug sequestration into subcellular particles\textsuperscript{8,30}. The second is due to action of multiple drug resistant proteins capable of active excretion of AEDs from the epileptogenic brains. They include P-gp, multidrug resistance proteins 1-5 (MRP 1-5), and lung resistance protein (LRP)\textsuperscript{33}. Since overexpression of Pgp has been reported in human epileptogenic brain, it suggests that P-gp may contribute to multiple drug resistance in epilepsy\textsuperscript{16,31}.

In the present study, moderate to strong P-gp immunoreactivity was observed in the vascular endothelium, dysplastic neurons, and many balloon cells, presented in the cortical layer I and in the subpial layer, whereas mild to moderate immunoreactivity for P-gp was noted in the vascular endothelium of normal brain. Not all balloon cells and abnormal dysplastic neurons were P-gp positive. More or less than 25\% of balloon cells and dysplastic neurons showed P-gp immunoreactivity in this study. Moreover, capillary endothelial cells in normal brain and a few normal-looking neurons from the cortical tuber lesions were also P-gp immunopositive. These findings suggested inducible expression of P-gp secondary to persistent and repetitive seizure attacks which demonstrated in an experimental epilepsy model\textsuperscript{17}.

P-gp is a member of the multidrug transporter family, and common antiepileptic drugs (AEDs), such as, carbamazepine, phenytoin, and valporate are substrates of the multidrug transporters\textsuperscript{23-25}. Thus, changes in the expressions of P-gp or MRPI may critically regulate the extracellular concentrations of AEDs and thus contribute to pharmacoresistance. The up-regulation of P-gp and MRPI has been reported only in NMD, including FCD, dysembryoplastic neuroepithelial tumors, and TSC\textsuperscript{12,27}. Recent studies have reported immunoreactivity for P-gp and MRPI on dysplastic neurons in 30-70\% of FCD cases\textsuperscript{26}, moderate to strong P-glycoprotein immunoreactivity in glial type balloon cells, and MRPI immunoreactivity in neuronal type balloon cells\textsuperscript{18}. We consider that upregulation of P-gp in balloon cells is more prevalent in TSC than FCD patients, and provides a plausible mechanism for medically refractory epilepsy in both TSC and FCD.

**Conclusion**

P-gp is the product of multidrug resistance gene (MDR1) and acts as an ATP-dependent membrane efflux transporter. It maintains intracellular drug concentration at a relatively low level. Overexpression of P-gp has been reported in human epileptogenic brain, it suggests that P-gp may contribute to multiple drug resistance in epilepsy. Changes in the expressions of P-gp or MRPI may critically regulate the extracellular concentrations of AEDs and thus contribute to pharmacoresistance. In this study, more or less than 25\% of balloon cells and dysplastic neurons showed P-gp immunoreactivity. Therefore, the drug expression resistance protein P-glycoprotein
in balloon cells and dysplastic neurons might explain medically refractory epilepsy in TSC.

• Acknowledgement
This study was financially supported by a research fund of Chonnam National University in 2004.

References
16. Loscher W, Potschka H: Role of multidrug transporters in pharmacoresistance to antiepileptic drugs. *J Pharmacol Exp Ther* 301: 7-14, 2002
31. Volkk H, Potschka H, Loscher W: Immunohistochemical localization of P-glycoprotein in rat brain and detection of its increased expression by seizures are sensitive to fixation and staining variables. *J Histochem Cytochem* 53: 577-581, 2005

Commentary

Authors presented an interesting result of up-regulation of drug resistance proteins in the brain of patients with tuberous sclerosis and intractable seizures. P-gp immunoreactivity in balloon cells and dysplastic neurons were 28.2% and 22.7%, respectively. These data can be a useful clue to elucidate the mechanism of drug-resistant epilepsy in congenital brain lesions such as tuberous sclerosis and neuronal migration disorders.

The major drawback of this paper is that their patients are consisted of only 2 patients treated surgically for epilepsy control and 6 autopsy cases whose cause of death or seizure history was not clearly described. Only the information from the data of two patients seems to be useful, because the data
from 6 autopsy series, even though they had seizures, lack the evidence that the tubers were responsible for their seizures.

Even in a single patient with tuberous sclerosis, some tubers are related with the patient's intractable seizures, while other tubers, at least temporarily, are not responsible for seizures. Valuable data should be derived from the tuber which had been proved to be related with drug-resistant intractable epilepsy in patients.

Despite this drawback, the authors showed very useful information by histopathological investigations in patients with tuberous sclerosis and intractable epilepsy. Demonstration of drug resistance gene expression in the diseased brain tissue will contribute to the elucidation of drug resistant epilepsy.

Seung-Chyl Hong, M.D., Ph.D.
Department of Neurosurgery
Samsung Medical Center