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Original article

A major influence of CYP2C19 genotype on the steady-state concentration of *N*-desmethylclobazam

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Abstract

N-desmethylclobazam (N-CLB), the major metabolite of clobazam (CLB), exerts a large influence on therapeutic and adverse effects of CLB. A substantial inter-individual variability has been observed in the ratios of N-CLB concentration/CLB dose and of the N-CLB/CLB concentration. We document here a genotype–phenotype correlation between CYP2C19 polymorphisms and those ratios. Patients with two mutated CYP2C19 alleles show significantly higher ratios than those with the wild type genotype: patients with one mutated allele exhibited intermediate trait. That is, the degree of elevation in the ratios was dependent on the number of mutated alleles of CYP2C19 (gene-dose effect). The N-CLB concentration/CLB dose ratio of patients with two mutated alleles was more than six fold higher than that of wild type patients. Thus, the serum N-CLB/CLB concentration ratio may be a valuable parameter to screen for patients at risk for side effects. Such precautions may be clinically relevant in populations where the mutant allele frequency is high, such as in Asian populations (~35%). Patients co-medicated with CYP3A4 inducer showed lower CLB concentration/CLB dose ratios and higher N-CLB/CLB concentration ratios. The overall effect of CYP3A4 inducer on N-CLB metabolism, however, was small and, thus, we conclude that the CYP2C19 genotype is the major determinant of the N-CLB concentration. For this reason it is crucial for the better management of epilepsy and other chronic illnesses in general to establish the correlation of genotype of CYP enzymes and pharmacokinetics/dynamics of drugs.

Keywords: Clobazam; Pharmacogenetics; CYP; P450; Benzodiazepine; Poor metabolizer

1. Introduction

Clobazam (CLB) is a benzodiazepine analog and has been widely used as an anti-epileptic drug (AED), often in combination with other AEDs. *N*-desmethylclobazam (N-CLB), the major metabolite of CLB, demonstrates a longer half-life, and much higher steady-state plasma concentration as compared to the parent drug CLB [1,2], exerting a larger influence on therapeutic and adverse effects of treatment [2,3]. Hence, it has been strongly recommended that the serum concentrations of not only the parent drug, CLB, but also the metabolite, N-CLB, be routinely measured. In this regard it is of note that a substantial inter-individual variability in the ratio of the serum N-CLB concentration/weight-adjusted CLB dose [4] and in the ratio of the serum N-CLB/CLB concentration has been observed with therapeutic drug monitoring.

The variability in the serum N-CLB concentration/CLB dose ratio and in the serum N-CLB/CLB concentration ratio has been partly accounted for by concurrent medication [5,6], especially in patients receiving drugs with phenobarbital, carbamazepine, and phenytoin [7]. Co-medication with those drugs induces CYP3A4, the major isoenzyme of the cytochrome P450 (CYP) enzyme family which plays a dominant role in the biotransformation of diverse drugs. CYP3A4 metabolizes CLB to N-CLB, and leads to the increased serum N-CLB/CLB concentration ratio. However, in patients who are not receiving CYP3A4 inducers, the serum N-CLB concentration is still not predictable from the initial CLB dose alone.

CYP2C19, another member of the CYP family is involved in the metabolism of several commonly prescribed

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drugs, including mephenytoin [8] and omeprazole [9]. It was first implicated in the metabolism of N-CLB based on the observation that the serum N-CLB concentration/CLB dose ratio and the serum N-CLB/CLB concentration ratio were significantly increased in patient(s) concurrently medicated with felbamate, an inhibitor of CYP2C19 [6]. Recently, Contin et al. [10] documented two patients with elevated serum N-CLB concentration/CLB dose ratios and N-CLB/CLB concentration ratios; one of these patients was homozygous and the other was heterozygous for the mutated allele of CYP2C19. The heterozygous patient was further reported in the recent issue of this journal [11].

It is known that in patients with CYP2C19 mutated alleles the metabolism of such drugs as mephenytoin and omeprazole is reduced [12]. A single base pair substitution (guanine to adenine) in exon 5 of CYP2C19 (the *2 variant according to the international nomenclature for the P450 alleles [13]) creates an aberrant splice site that alters the reading frame of the mRNA, starting with amino acid 215, and produces a premature stop codon 20 amino acids downstream [14]. A single base pair mutation (guanine to adenine) at position 636 in exon 4 of CYP2C19 (the *3 variant according to the international nomenclature), also results in a premature stop codon [15]. Hence, both CYP2C19*2 and *3 alleles result in a truncated, non-functional protein.

There are inter-racial differences in the allele frequency of the *2 and *3 alleles of CYP2C19. The combined frequency of *2 and *3 alleles is much higher in the Japanese population (35%) [15–17] than in the Caucasian population (20%). Furthermore, the *2 and *3 alleles account for almost all of the individuals with the 'poor-metabolizer' phenotype in the Japanese population [15–17]. Giving careful consideration to such a unique population structure among Japanese, we tested whether the CYP2C19 genotype was associated with an altered metabolism of CLB and N-CLB among Japanese patients receiving CLB for the treatment of epilepsy.

2. Subjects and methods

A group of 16 patients, between the ages of 1.5 and 33 years (median, 7 years), were recruited from the Neuropediatrics Clinic at the Keio University Hospital (Table 1). All patients had received oral administration of tablets or granules of CLB (Mystan; Dainippon, Co. Ltd, Osaka, Japan) with the dose unchanged at least for 4 weeks at the time of study. In 6 out of 16 patients, the underlying neurological conditions were documented: these included subdural hematoma, intracerebral hemorrhage, meningoencephalitis, megalencephaly, neurofibromatosis, and brain abscess. All the patients were concomitantly receiving other AEDs. Seven patients were receiving at least one AED(s) with CYP3A4-inducing properties (inducers: carbamazepine, phenytoin, and phenobarbital), whereas 9 patients were receiving only anti-epileptic drug(s) without CYP3A4-inducing properties (non-inducers: valproic acid, ethosuximide, and zonisamide) [7]. Serum concentrations of CLB and N-CLB were measured using gas chromatography-mass spectrometry [16]. We assumed that the spot concentrations represented the steady-state concentrations [4] because the steady state is reached within about 4 days of repeated doses for CLB and within 10 days for N-CLB [1]. The study protocol was approved by the institutional review board, and all the participants or the parent(s) of the participants provided their written informed consent.

Genomic DNA was isolated from whole blood samples using the QIAamp Blood Kit (QIAGEN, Hilden, Germany), and CYP2C19*2 and CYP2C19*3 mutations were detected by polymerase chain reaction using previously described primers [17]. The amplified fragments were analyzed using either an autosequencer (ABI PRISM 3100, Applied Biosystems) or a denaturing high-performance liquid chromatography (DHPLC) system (Transgenomic, Omaha, NE) using a previously described method [18]. Since both the CYP2C19*2 and *3 alleles abolish protein

Table 1
Demographic characteristics of patients grouped according to CYP2C19 genotype

	Group 1 ($n = 7$)	Group 2 ($n = 6$)	Group 3 ($n = 3$)	Significance*
Age (year)				
Mean	6.79	12.2	14.3	
Range	1.5–14	4–22	5–33	
Male/female	4/3	0/6	2/1	NS
Body weight (kg)#	22.8 ± 10.7	33.7 ± 15.2	36.7 ± 32.5	NS
Dose (mg/kg per day)#	0.28 ± 0.19	0.32 ± 0.19	0.39 ± 0.26	NS
C_{ss} (ng/ml)#				
CLB	130 ± 130	219 ± 196	168 ± 96	NS
N-CLB	586 ± 459	2015 ± 1333	4806 ± 2575	$P < 0.05$†

Patients are classified according to the total count of mutated allele(s) into three groups (abscissa): group 1, CYP2C19 *1/*1; group 2, CYP2C19 *1/*2 or CYP2C19 *1/*3; group 3, CYP2C19 *2/*2, CYP2C19 *3/*3, or CYP2C19 *2/*3. C_{ss}, steady-state serum concentration; NS, not significant.

* Significance determined by Tukey-Kramer or Fisher exact test.

\# Data are expressed as mean ± standard deviation.

† Comparison between groups 1–2 and groups 1–3.

function [14,15], their contributions to the poor-metabolizer phenotype were expected to be comparable.

We classified the patients according to the total count of mutated allele(s): group 1, CYP2C19 *1/*1; group 2, CYP2C19 *1/*2 or CYP2C19 *1/*3; group 3, CYP2C19 *2/*2, CYP2C19 *3/*3, or CYP2C19 *2/*3. The patients were also cross-classified based on the presence or absence of a CYP3A4-inducer co-medication. Differences in the response variables including the CLB concentration/CLB dose ratio, the NCLB concentration/CLB dose ratio, and the N-CLB/CLB concentration ratio among the groups were then evaluated using a two-way ANOVA with more than one observation per cell (S-plus, Insightful, USA).

3. Results

The number of patients with each genotype was as follows: CYP2C19 *1/*1, $n = 7$; CYP2C19 *1/*2, $n = 4$; CYP2C19 *1/*3, $n = 2$; CYP2C19 *2/*2, $n = 2$; CYP2C19 *3/*3, $n = 0$; CYP2C19 *2/*3, $n = 1$. Hence, there were 7 patients in group 1 (*1 homozygotes), 6 patients in group 2 (heterozygotes for *2 or *3), and 3 patients in group 3 (homozygotes for *2 or compound heterozygotes for *2 and *3). The background characteristics (age, sex, weight, dose/weight) were comparable among the three groups (summarized in Table 1).

The serum concentrations of CLB and N-CLB (ng/ml) adjusted for the daily dose of CLB per body weight (mg/kg per day) were compared among the three groups (Fig. 1). The mean values for the CLB concentration/CLB dose ratios in groups 1, 2, and 3 were 443, 819, and 457 ng/ml per mg/kg/day, respectively (Fig. 1A). The mean values for the N-CLB concentration/CLB dose ratios in groups 1, 2, and 3 were 2111, 7156, and 13,504 ng/ml per mg/kg/day, respectively (Fig. 1B). Thus, the effect of the number of mutated allele(s) of CYP2C19 (a gene-dose effect) on the N-CLB concentration/CLB dose ratio was robust whereas such an effect was not observed with respect to the CLB concentration/CLB dose ratio. The N-CLB/CLB concentration ratio increased as the number of mutated allele(s) increased, again suggestive of a gene-dose effect (Fig. 2).

Within each of groups 1–3, patients receiving the CYP3A4-inducer co-medication tended to have a lower CLB concentration/CLB dose ratio (Fig. 1A), a lower N-CLB concentration/CLB dose ratio (Fig. 1B) and a higher N-CLB/CLB concentration ratio than those not receiving CYP3A4-inducer (Fig. 2). Thus, we have evaluated differences in the ratios (CLB concentration/CLB dose, N-CLB concentration/CLB dose, N-CLB/CLB concentration) between patients receiving inducer and those not: a two-way ANOVA was applied by cross-classifying each of the three groups in two-ways, based on the presence or absence of CYP3A4-inducer co-medication. The CLB concentration/CLB dose ratio was invariable and independent of the genotype category ($P = 0.63$) but significantly decreased in the presence of CYP3A4-inducing co-medication ($P = 0.005$). The N-CLB concentration/CLB dose ratio was strongly dependent upon the genotype category

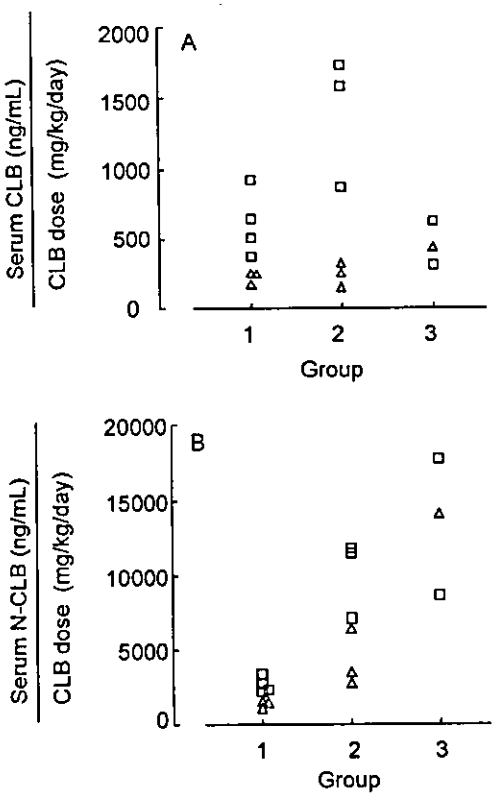

Fig. 1. Gene-dose effect of CYP2C19 polymorphism on the serum CLB concentration/CLB dose ratio (A) and on the serum N-CLB concentration/CLB dose ratio (B). Patients are classified according to the total count of mutated allele(s) into three groups (abscissa): group 1, CYP2C19 *1/*1; group 2, CYP2C19 *1/*2 or CYP2C19 *1/*3; group 3, CYP2C19 *2/*2, CYP2C19 *3/*3, or CYP2C19 *2/*3. △, Patients receiving CYP3A4-inducing co-medication(s); □, patients receiving CYP3A4-non-inducing co-medication(s).

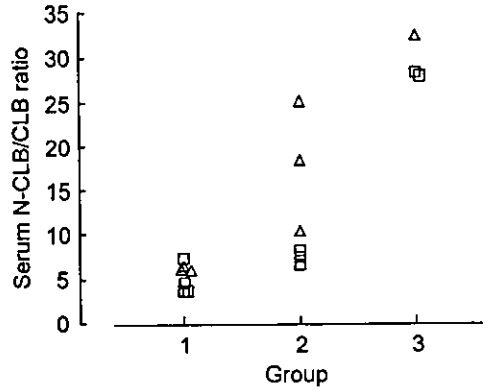

Fig. 2. Gene-dose effect of CYP2C19 polymorphism on the serum N-CLB/CLB concentration ratio. Patients are classified according to the total count of mutated allele(s) into three groups (abscissa): group 1, CYP2C19 *1/*1; group 2, CYP2C19 *1/*2 or CYP2C19 *1/*3; group 3, CYP2C19 *2/*2, CYP2C19 *3/*3, or CYP2C19 *2/*3. △, Patients receiving CYP3A4-inducing co-medication(s); □, patients receiving CYP3A4-non-inducing co-medication(s).

($P < 0.0001$) and significantly decreased in the presence of CYP3A4-inducing co-medication ($P = 0.01$). The N-CLB/CLB concentration ratio was strongly dependent upon the genotype category ($P < 0.0001$) and increased in the presence of CYP3A4-inducing co-medication ($P = 0.04$).

4. Discussion

In the present study, we documented a genotype–phenotype correlation between two mutated CYP2C19 polymorphisms and the N-CLB concentration/CLB dose ratio as well as the N-CLB/CLB concentration ratio. Those ratios in the patients with two mutated CYP2C19 alleles (group 3 in this study) were significantly higher than those in patients with the wild type genotype (group 1). Patients with one mutated allele (group 2) exhibited an intermediate trait. These observations give further credence to the notion that CYP2C19 is involved in the degradation of N-CLB [10].

When the patients in each group were cross-classified based on the presence or absence of CYP3A4-inducing co-medication, patients co-medicated with CYP3A4 inducer tended to have lower CLB concentration/CLB dose ratios and higher N-CLB/CLB concentration ratios. These changes in the two ratios are most likely to be due to elevated expression of CYP3A4 which converts CLB to N-CLB. However, the overall effect of a CYP3A4 inducer on N-CLB metabolism was smaller than that of CYP2C19 polymorphism. We conclude that CYP2C19 genotype is the major determinant of the steady-state concentration of N-CLB.

From a clinical standpoint, it is important to note that the mean values for the N-CLB concentration/CLB dose ratio of patients with two mutated alleles (group 3) were more than six fold higher than those of wild type patients (group 1). Although anti-epileptic potency of N-CLB has been reported to be one-fourth of that of CLB [19,20], it is highly likely that patients with two mutated alleles are susceptible to a higher incidence of side effects given a significant increase in the N-CLB concentration in group 3. Indeed, while receiving a standard dosage of CLB, one of the patients with two mutated alleles experienced excessive somnolence, one of the common adverse effects of CLB. Thus, a large-scale prospective study is warranted to determine whether homozygosity for a CYP2C19 mutation predisposes an individual to a higher incidence of side effects.

The serum N-CLB/CLB concentration ratio may be a valuable parameter for detecting patients with CYP2C19 polymorphisms: All three patients with two mutated CYP2C19 alleles (group 3) had N-CLB/CLB concentration ratios of 25 or more. We recommend that the N-CLB/CLB concentration ratio be closely monitored at the initial phase of CLB therapy in order to screen patients with two mutated alleles (group 3) who may be at risk for developing side effects if the standard dose of CLB is prescribed. Such precautions may be clinically relevant in populations where the mutated allele frequency is high, such as in Asian populations (15–20%). In interpreting the N-CLB/CLB concentration ratio, it should be noted that in those patients taking CYP3A4-inducing co-medication the ratio may be elevated in heterozygotes (group 2) as well.

The degree of elevation in the N-CLB concentration/CLB dose ratio and that in the N-CLB/CLB concentration ratio were dependent on the number of mutated alleles of CYP2C19. This gene-dose effect recapitulates observations for omperazole, a proton pump inhibitor which also is metabolized by CYP2C19 [12]. Omperazole produces a greater cure rate for gastric ulcers and accompanying *Helicobacter pylori* infections in patients with heterozygous as well as homozygous mutations of CYP2C19 because blood levels are higher in these individuals [21]. Therefore, it is likely that not only the blood concentration of N-CLB (established in this study) but also the efficacy of the drug for the treatment of epilepsy (both therapeutic and adverse effects) is gene-dose dependent. Studies with a more homogeneous patient population with a larger number of patients enrolled will allow such an assessment.

The limitations of the present study are two-fold. First, the CLB and N-CLB concentrations were measured only at a single time point, as opposed to multiple points. However, all the patients had been receiving CLB for much longer than the periods required to reach a steady-state levels of CLB and N-CLB [1]. Thus, it is assumed that a single data point is likely to represent the steady-state concentration. A formal population pharmacokinetic evaluation [22] would allow the N-CLB concentration to be predicted based on the patient's genotype. Second, the patients were only screened for the *2 and *3 alleles. Hence, some of the *2 or *3 heterozygotes who were not receiving CYP3A4 inducers but who had elevated N-CLB concentration/CLB concentration ratios may have actually been compound heterozygotes. However, the rarity of mutated alleles other than *2 and *3 in the Japanese population makes such concerns merely theoretical. When studying other ethnic groups, however, screening for other mutated alleles may be necessary, considering that several new alleles of CYP2C19 that induce coding changes were recently identified [23].

Our data indicate that three different patient groups (wild-type, heterozygotes for the mutated alleles, and mutation homozygotes or compound heterozygotes) may respond to CLB in distinctive manners. In future evaluations of new AEDs, the genotype of the subject should be considered as a critical confounding factor when the drug under development, or its active metabolite, is targeted by a highly polymorphic drug metabolizing enzyme, such as in the presently reported situation with CYP2C19. Furthermore, the results of clinical trials performed in one ethnic group or country should be carefully interpreted when

applied to another group or country because the gene frequency of the mutated allele(s) can vary significantly among different ethnic groups. Lastly we emphasize that it is of crucial clinical importance for the better management of epilepsy and other chronic illnesses in general to establish the correlation of genotype of CYP enzymes and pharmacokinetics/dynamics of drugs.

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1．けいれんの診療

3．けいれん時の脳代謝

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Key words：グルタミン酸，NMDA，カルシウムイオン

けいれんによる過剰な神経電気活動により，神経細胞の代謝は異常亢進する。通常，代謝の増加に見合うだけの血流増加がみられるが，長時間にわたるけいれん（けいれん重積）では代謝と血流の不均衡を生じ，不可逆的な脳傷害をきたす可能性がある。

一方，低酸素血症や低血糖，局所循環傷害がなくとも，けいれん重積により神経組織の傷害をきたすことが知られている。これらの組織傷害では傷害の起こるタイミングおよび病理組織像が低酸素脳症や低血糖による脳傷害のそれとは異なっている。すなわち，過剰な神経電気活動そのものが脳傷害の原因となっていると考えられている。その機序として，とくに興奮性アミノ酸の過剰刺激による神経細胞傷害が重要である。けいれん重積後に知能障害やてんかんが発症する機序としても注目されている。

けいれん時代謝性脳傷害の発症機序のひとつに神経細胞質内のカルシウム濃度調節異常があげられる。神経細胞質内のカルシウム濃度は，ミトコンドリアや小胞体への取り込み，細胞外への能動輸送などによって，細胞外の1/10,000程度に保たれている。けいれん重積時には，エネルギー依存性カルシウム能動輸送が障害されることによって神経細胞傷害が生じると考えられている。

脳の電気活動の約半分は，グルタミン酸やアスパラギン酸などの興奮性アミノ酸を神経伝達物質とするニューロンによって行われている。けいれん時には，興奮性アミノ酸による過剰刺激により大量のナトリウムとカルシウムが神経細胞内に流入，アポトーシスなどの機序を介して細胞傷害をきたす。興奮性アミノ酸の中で，けいれん時脳代謝と関連してとくに重要なのがグルタミン酸である。

興奮性ニューロンの神経末端から放出されたグルタミン酸は，シナプス間隙から能動輸送によって速やかに細胞内に再吸収されるため，細胞外グルタミン酸濃度は非常に低濃度に維持されている。低酸素血症や低血糖などにおいてはグルタミン酸の能動輸送が障害されることによって細胞外グルタミン酸濃度が上昇し，神経細胞傷害を助長すると考えられている。

substance P，galaninなどの神経伝達物質の変動もグルタミン酸の放出に影響し，けいれんの惹起・持続に関与すると考えられている[1]。すなわち，substance P，neurokinin Bはグルタミン酸の放出を促進する。galaninはシナプス前膜に作用して過分極を起こし，グルタミン酸放出を抑制，NMDA受容体の作用を抑える。dynorphinもgalaninと同様な作用を有する。けいれん重積時にはgalaninが枯渇し，次第に薬剤耐性を生じる。けいれんが長時間に及んだ場合に抗てんかん薬に対する反応性が低下する現象に関与している可能性がある。

グルタミン酸受容体として，N-methyl-D-aspartate（NMDA）受容体，α-amino-3-hydroxy-5-methyl-4-isoxazole（AMPA）受容体，kainate（KA）受容体，数種類のmetabotropic受容体が知られており[2]（表1の1），それぞ

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表 1　グルタミン酸受容体とサブユニット

1．グルタミン酸受容体
　a）イオンチャンネル（Na および Ca チャンネル）に連結
　　・NMDA 受容体
　　・AMPA 受容体
　　・KA 受容体
　b）metabotropic 受容体
　　・L-AP 4 受容体
　　・ACPD 受容体
　　・その他
2．サブユニット構成
　a）NMDA 受容体サブユニット（4 量体）
　　・NR1
　　・NR 2 A，NR 2 B，NR 2 C，NR 2 D（それぞれ NR 1 との組み合わせで発現）
　b）AMPA 受容体サブユニット
　　・GluR1，GluR 2，GluR 3，GluR 4
　c）KA 受容体サブユニット
　　・GluR 5，GluR 6，KA1，KA 2
　d）metabotropic 受容体（L-AP 4 受容体，ACPD 受容体など）
　　グループ I（mGluR1，mGluR 5）
　　Gq シグナル系：protein kinase C を介し，phosphatidylinositol 4,5-bisphosphate 水酸化促進
　　シナプス後膜に存在，神経興奮閾値低下
　　グループ II（mGluR 2，mGluR 3）およびグループ III（mGluR 4，mGluR 6，mGluR 7，mGluR 8）
　　Gi/Go シグナル系：adenylate cyclase を抑制，シナプス前膜に存在，神経伝達物質放出を抑制

（Baudry ら[2]，2001）

表 2　成長やけいれんに伴う受容体の変化

グルタミン酸受容体サブユニットの変化
1．年齢による違い（Sanchez ら）[3]
NMDA 受容体：新生時期では NR 2 B が NR 2 A に比べて多い。NR 2 B を主体とする NMDA 受容体では NR 2 A を主体とする受容体に比べて興奮持続時間が長く，細胞内への Ca 流入が多い。
AMPA/KA 受容体：新生時期では大脳皮質・海馬で GluR 2 が少ない。GluR 2 を欠く AMPA/KA 受容体では Ca 透過性が比較的高い。
2．けいれん後の変化（Treiman ら）[4]
海馬における mGluR 2/3，mGluR 5 の増加
海馬 CA1，CA 3 領域における GluR 2 の減少

れの受容体はいくつかのサブユニットの組み合わせによって構成されている（表 1 の 2）。NMDA 受容体については，グリシン結合部位，Na，Ca イオンチャンネル，Mg 結合部位などが解明されている。

グルタミン酸受容体を構成するサブユニット構成は年齢特異的であり，また，けいれんの結果変動することが知られている（表 2）[3,4]。けいれんによる神経損傷の程度や抗てんかん薬の有効性が年齢依存性である理由のひとつとして，年齢によるサブユニット構成の違いがあげられている。

けいれんによる過剰な神経電気活動時には，イオンチャンネルに連結した NMDA 受容体や AMPA/KA 受容体を介して細胞外 Ca^{++} が細胞内に流入する。また，L-AP 4 受容体や ACPD 受容体などの metabotropic 受容体においては phosphatidylinositol 4,5-bisphosphate/protein kinase C 系を介して Ca^{++} が小胞体より細胞質内へ放出される。通常は，エネルギー依存性ポンプによる Ca^{++} の細胞外への排出および Ca/Mg ATPase を介する小胞体への取り込み促進によって細胞質内 Ca^{++} 濃度は適正に保たれている。しかし，低血糖や虚血においてはエネルギー不足により細胞質内 Ca^{++} 濃度が上昇，さらに活性酸素やフリーラジカルなどが関与して細胞傷害が生じると考えられている[5]。

Ca^{++} による細胞傷害の発生機序については専門書を参照されたい。

まとめ

けいれん時に起こる広義の脳代謝異常，すなわち低酸素血症などによるエネルギー異常，および特異的代謝異常，すなわち興奮性アミノ酸の過剰による細胞質内イオン，とくに Ca^{++} 濃度の変動と神経細胞傷害について簡単にまとめた。とくに，グルタミン酸過剰刺激による神経細胞傷害については，けいれんに続発する脳障害を予防するために今後さらなる検討が進められると思われる。

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Case report

Acute dysautonomia: complete recovery after two courses of IVIg

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Abstract

An 11-year-old boy presented with acute dysautonomia manifesting as severe orthostatic hypotension following fever. Serial orthostatic tests with measurement of the coefficient of variation in the R–R intervals showed improvement after one course and complete recovery after two courses of intravenous high-dose immunoglobulin therapy (IVIg). Repeated courses of IVIg should be considered to treat this disorder if spontaneous remission does not occur.

Keywords: Acute dysautonomia; Orthostatic hypotension; Intravenous high-dose immunoglobulin therapy

Acute dysautonomia is a rare disorder, characterized by acute dysfunction of the autonomic nervous system with a history of preceding infection [1,2]. Acute dysautonomia has recently been recognized as a type of neuroimmunologic disorder. The pathogenesis remains unclear, but may involve acute inflammatory neuropathy caused by an immune mediated mechanism, similar to Guillain–Barré syndrome [2,3]. Recovery from acute dysautonomia tends to be gradual and frequently incomplete, and early therapeutic intervention is necessary in patients with progressive disability [1,2]. Acute dysautonomia has been successfully treated with intravenous high-dose immunoglobulin therapy (IVIg) [4–8]. We treated an 11-year-old boy who developed severe orthostatic hypotension after an episode of fever and recovered after two courses of IVIg.

1. Case report

A previously healthy 11-year-old boy developed high fever of 40 °C in September 2001 (Fig. 1). He complained of headache and dizziness at that time. His condition was treated as a common cold. The fever ceased 3 days later, but he suffered from sustained dizziness. Blood tests showed no abnormalities, but his blood pressure decreased from 88/46 mmHg to 68/42 mmHg at 4 min after standing. The diagnosis was orthostatic hypotension. He was treated with vasopressors, anti-inflammatory drugs, antidepressants, etc., but with no effect. Two months after the onset, he was referred to our hospital for further evaluation and treatment.

Serial measurements of the coefficient of variation in the R–R intervals (CVR–R) were made using a multi-function electrocardiograph (FDX-4520; Fukuda Denshi, Japan) during the orthostatic test monitored with a continuous non-invasive blood pressure tonometry system (JENTOW-7700; Colin Corporation, Japan). The orthostatic test was performed after resting for more than 15 min, and systolic and diastolic blood pressures were recorded every 10 to 15 s. The CVR–R was also measured at rest.

The orthostatic test demonstrated severe orthostatic hypotension (Fig. 2a). Immediately after standing, his heart rate suddenly rose to 140 beats/min, and his systolic blood pressure to higher than 140 mmHg. Seven minutes later, the systolic blood pressure sharply fell to lower than 80 mmHg, and he felt dizziness and loss of vision, and was unable to remain standing. He then lay down, but his heart rate and blood pressure did not recover to those measured before standing for 13 min. CVR–R was severely low at 2.05% (normal lower limit 3.0% in control subjects aged 10–19 years old) (Fig. 1).

He had a good appetite, and had no constipation or diarrhea. His hands were always cold and wet, but physical examination demonstrated no other abnormalities. He had no

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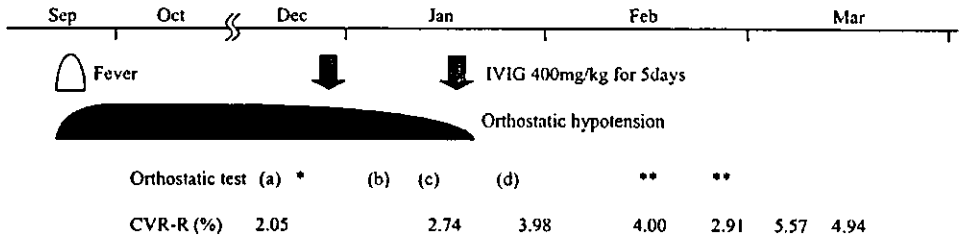

Fig. 1. Clinical course and timing of orthostatic tests and treatment. Coefficient of variation in the R–R intervals (CVR–R) showed gradual improvement after two courses of intravenous high-dose immunoglobulin therapy. As for orthostatic tests, * showed similar pattern to (a), and ** to (d). Refer to the results of orthostatic tests in Fig. 2.

vesicorectal problems. Neurological examination also demonstrated no abnormalities, including the cranial nerves, motor and sensory systems, and deep tendon reflexes. The ocular fundi were also normal. Motor and sensory nerve conducting velocities in the median nerves were within the normal range. Metaiodobenzylguanidine scintigraphy, which utilizes the noradrenaline analog metaiodobenzylguanidine for detection of autonomic dysfunction in the heart, showed no abnormalities. No abnormalities were found in blood and urine tests, including serum levels of antiganglioside antibodies. Cerebrospinal fluid (CSF) testing on admission showed normal levels of protein (18 mg/dl) and cell count (1/μl). Blood examination showed that immunoglobulin G antibody for human herpes virus 6 (HHV-6) was highly positive (1:160, normal < 1:10), and immunoglobulin M antibody for HHV-6 and polymerase chain reaction amplification of HHV-6 deoxyribonucleic acid were negative. Other virus antibody titers were negative.

The diagnosis was acute dysautonomia. He was treated with IVIg (400 mg/kg body weight for five consecutive

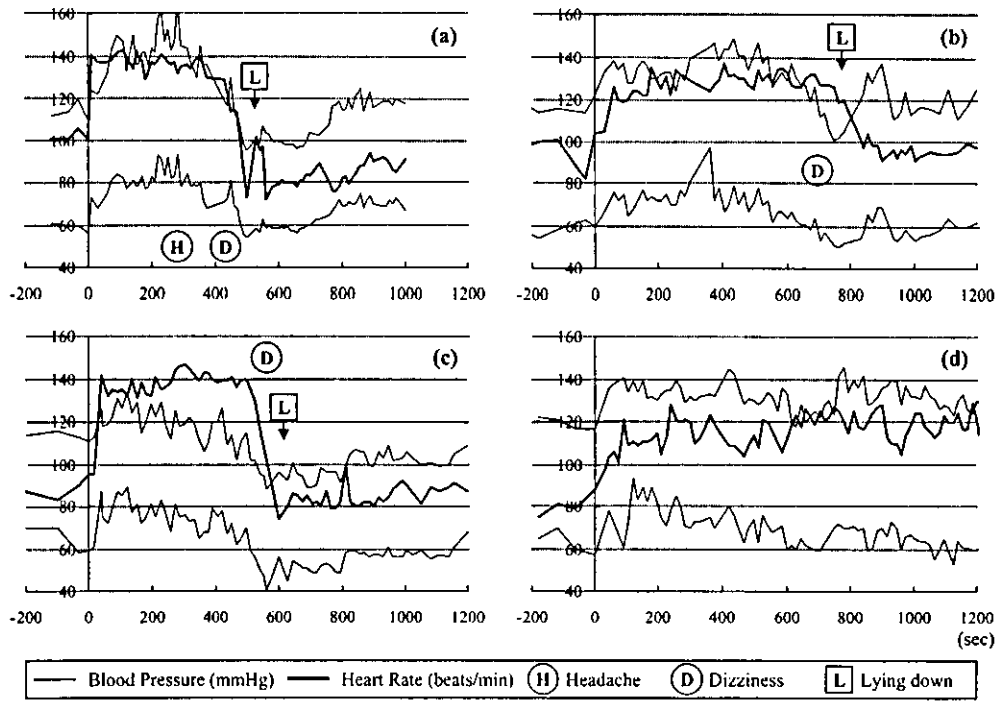

Fig. 2. Results of orthostatic tests. The timing of orthostatic tests was shown in Fig. 1. (a) Before treatment, the orthostatic test showed severe orthostatic hypotension, with sudden rises in blood pressure and heart rate immediately after standing, followed by impulsive falls in both 7 min after standing. Recovery of blood pressure and heart rate was poor after lying down. (b) The orthostatic test showed slight improvement after one course of intravenous high-dose immunoglobulin therapy, when he could remain standing for as long as 13 min. Changes in blood pressure and heart rate were less severe than before treatment, and recovery after lying down was prompt. (c) One week later, the orthostatic test showed a similar pattern to that before treatment. (d) The orthostatic test showed complete recovery after a second course of treatment.

days). The orthostatic test then showed his condition had slightly improved (Fig. 2b). He could remain standing for as long as 13 min. The changes in blood pressure and heart rate were less severe than before treatment, and recovery after lying down was prompt. However, one week later, the orthostatic test showed similar results to those before treatment (Fig. 2c), and CVR–R was still abnormally low at 2.74%. Another course of IVIg was given. The orthostatic test showed complete recovery (Fig. 2d) and the CVR–R returned to normal values (3.98%) (Fig. 1). He has had no complaints for more than one year.

2. Discussion

Acute dysautonomia can be considered as an uncommon variant of Guillain–Barré syndrome [5–8], because of the relatively common association of autonomic symptoms in Guillain–Barré syndrome, and because of the elevation of CSF protein in acute dysautonomia [6–8]. The level of CSF protein in our patient was normal, but the absence of any abnormality might be due to the late timing of the CSF examination, two months after the onset.

Our patient required two courses of IVIg to resolve his symptoms. The orthostatic test showed only slight improvement after the first course. Only one week later, the orthostatic test showed worsening of the symptoms and abnormally low CVR–R. However, temporary improvement was apparently observed after the first course, and a few reports have suggested that two courses of IVIg are necessary for effectiveness in cases of Guillain–Barré syndrome [9,10]. He showed complete and permanent recovery after the second course of treatment. The present case indicates that a second course of IVIg should be considered in patients with severe acute dysautonomia, especially if signs of improvement are observed after the first course.

Serial measurements of CVR–R and the orthostatic test using a tonometry system were useful for the evaluation of autonomic function in a child with acute dysautonomia. These methods are non-invasive and easy to carry out even in pediatric patients.

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Clinical efficacy of zonisamide in childhood epilepsy after long-term treatment: a postmarketing, multi-institutional survey

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KEYWORDS
Zonisamide;
Epilepsy;
Japan;
Pediatric;
Efficacy

Summary Postmarketing data about the effectiveness of zonisamide in childhood epilepsy was collected from 759 children with various forms of epilepsy (ages 3 months–15 years) to compare the long-term efficacy of zonisamide in the treatment of epilepsy in intellectually normal versus intellectually disabled children. The follow-up period was 6 months–3 years; 291 children (245 intellectually normal, 46 intellectually disabled) received zonisamide as monotherapy. The remaining patients received additional antiepilepsy drugs (AEDs); mean numbers of additional AEDs were 1.6 and 2.9 for intellectually normal and intellectually disabled groups, respectively. Effectiveness could not be evaluated in 30 of the 759 patients because of very rare or irregular seizure frequency. In the 729 patients evaluated, 78% of intellectually normal patients and 43% of intellectually disabled patients showed $\geq$50% reduction in the number of seizures ($P < 0.001$). Improvement rates seen in the intellectually normal group were almost the same for patients with generalized (82%) and partial (77%) epilepsies, whereas in the intellectually disabled group, the improvement rate was higher for partial (50%) than generalized (36%) epilepsies ($P < 0.01$).

These results are consistent with the known phenomenon that intellectually disabled children are likely to have more intractable seizures than children with normal intelligence.

Introduction

Zonisamide is a new drug with broad-spectrum antiepilepsy activity against partial as well as generalized seizures. The drug has been commercially licensed in Japan since 1989. Before licensure, randomized, controlled trials of zonisamide were performed by 4 authors;[1–4] 3 studies were of treatment in adults,[1–3] and 1 study examined pediatric treatment.[4] The observation period of these studies was 12–16 weeks in adults, and 8 weeks in children. All studies were confined to zonisamide treatment of generalized seizures. Postmarketing research, performed at 25 institutions over a 5-year period, investigated the clinical effectiveness and safety of zonisamide for the treatment of childhood epilepsy. The purpose of this postmarketing study was to compare the long-term efficacy of zonisamide in the treatment of epilepsy in intellectually nor-

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mal children to that in intellectually disabled children.

Methods

Patients and study design

A total of 759 patients were included in the study. All were under 15 years of age. Ages of patients distributed as follows: less than 1 year, 20 patients (3%); 1–5 years, 213 patients (28%); 6–10 years, 282 patients (37%); and 11–15 years, 244 patients (32%). Of the study subjects, 418 were evaluated as intellectually normal, and 341 were intellectually disabled, with scores less than 50 based on their intelligent quotient (IQ) or development quotient (DQ). The IQ was evaluated by Tanaka-Binet method (Japanese edition of the Stanford-Binet Intelligence Scale), and in a few cases, by Wechsler Intelligence Scale for Children—Revised. The DQ was evaluated by the Tsumori-Inage method and Enjoji method (both are evaluated by interviewing parents, similar to the Denver Developmental Screening Test). The observation period of the study was between 6 months and 5 years.

Overall improvement in seizure control was evaluated in both the intellectually normal and the intellectually disabled groups. In the intellectually normal group, approximately 15% of patients had temporal lobe epilepsy and approximately 55% had non-temporal lobe epilepsy. Generalized epilepsies were found in less than 20% of this group. In contrast, in intellectually disabled patients, approximately 7% had temporal lobe epilepsy and 35% non-temporal lobe epilepsy. Generalized epilepsy was found in almost one-half of this study group's subjects.

A total of 729 patients out of the initial 759 patients were included in the final evaluation. Thirty patients were eliminated from analysis because of loss to follow-up due to adverse effects, change of address, too few seizures to evaluate, or other reasons. Of the intellectually normal group, 401 patients completed the study; of the intellectually disabled group, 328 completed the study (Table 1).

Table 2 Number of AEDs.

Number of AEDs	Number of patients
Intellectually normal (mean 1.6)	
Zonisamide monotherapy	245 (61%)
2 AEDs	112 (28%)
3 AEDs	44 (11%)
4 AEDs	12 (3%)
Intellectually disabled (mean 2.9)	
Zonisamide monotherapy	46 (14%)
2 AEDs	67 (20%)
3 AEDs	128 (39%)
4 AEDs	64 (20%)
5 AEDs	32 (10%)

Evaluation of safety and efficacy

The efficacy of zonisamide in the treatment of seizures was evaluated on the basis of improvement over a minimum 5-month period of observation following the initiation of treatment. Patients who were observed less than 5 months were not included in the efficacy analysis. Statistical analysis was performed using the chi-square method. Doses of zonisamide varied from 2 mg/kg per day to more than 10 mg/kg per day, and about 70% of patients received 2–8 mg/kg per day of zonisamide.

Zonisamide was regarded as effective for seizure control if the seizure frequency was reduced by $\geq$50% compared to baseline seizure frequency measured before treatment began. The improvement rate was defined as the percentage of the total number of patients meeting this efficacy standard. Adverse effects were recorded for all patients.

Sixty-one percent of the patients in the intellectually normal group were treated with zonisamide monotherapy (Table 2). Twenty-eight percent of the patients were treated with zonisamide and 1 other antiepilepsy drug. In contrast, zonisamide monotherapy was given to only 14% of the patients in the intellectually disabled group. Zonisamide was given together with 2 other antiepilepsy drugs

Table 1 Number of patients (final evaluation).

	Epilepsies			Total
	Generalized	Partial	Unclassified	
Intellectually normal	67	331	3	401
Intellectually disabled	152	170	6	328
Total	219	501	9	729

Thirty patients were excluded because of interruption of follow-up by adverse effects, changes of address, etc.

in 39% of this group's patients. The mean number of AEDs, including zonisamide, was 1.61 in the intellectually normal group and 2.94 in the intellectually disabled group.

Results

Efficacy

The overall improvement rating for generalized seizures is shown in Table 3. The characteristics of the seizures were different for the 2 groups. Generally speaking, improvement was significantly beneficial for persons of normal intelligence (80%, $P < 0.001$), notably for tonic (89%, $P < 0.001$), and generalized tonic–clonic seizures (87%, $P < 0.01$), as well as other seizure types (80%, $P < 0.05$). For all categories of partial seizures, the improvement rating was significantly higher ($P < 0.001$) in the intellectually normal group (86%) than in the intellectually disabled group (64%) (Table 4).

Table 3 Overall improvement rating: generalized seizures.

Seizure type	Improved (reduction ≥50% of seizure frequency)	
	Intellectually normal	Intellectually disabled
Absence	3/4 (75%)	None
Atypical absence	4/8 (50%)	6/19 (32%)
Myoclonic seizure	3/6 (50%)	23/43 (53%)
Tonic seizure	16/18 (89%)***	28/77 (36%)
GTC	26/30 (87%)**	21/37 (57%)
Others	19/23 (83%)*	14/28 (50%)
Total	71/89 (80%)***	92/204 (45%)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ between the intellectually normal and the intellectually disabled groups, respectively; GTC = generalized tonic–clonic.

Table 4 Overall improvement rating: partial seizures.

Seizure type	Improved (reduction ≥50% of seizure frequency)	
	Intellectually normal	Intellectually disabled
SPS	73/89 (82%)***	27/39 (69%)
CPS	146/170 (86%)***	74/123 (60%)
SGS	131/150 (87%)***	70/103 (68%)
Others	11/13 (85%)***	3/5 (60%)
Total	361/422 (86%)***	174/270 (64%)

SPS = simple partial seizure; CPS = complex partial seizure; SGS = secondarily generalized seizure.
***$P < 0.001$ between the intellectually normal and intellectually disabled groups.

Table 5 compares the improvement rating according to the classification of epilepsy syndromes. The improvement rating was higher ($P < 0.001$) in the intellectually normal group (78%) than in the intellectually disabled group (43%). In the intellectually disabled group, patients with partial epilepsy showed a slightly better response than those with generalized epilepsy (50% versus 36%, $P < 0.01$).

Table 6 shows the improvement rating for patients with various types of generalized epilepsies. Primary generalized epilepsy was better controlled by zonisamide ($P < 0.001$) in intellectually normal patients (91%) than in intellectually disabled patients (58%). The improvement rating data for partial epilepsies is shown in Table 7. Only temporal lobe epilepsy did not show a significant difference in the improvement rating between the 2 groups.

Table 5 Overall improvement rating.

	Syndrome	Improved	Unimproved	Total
Intellectually normal	Generalized	55 (82%)***	12 (18%)	67 (100%)
	Partial	256 (77%)	75 (23%)	331 (100%)
	Unclassified	3 (100%)	0 (0%)	3 (100%)
	Total	314 (78%)***	87 (22%)	401 (100%)
Intellectually disabled	Generalized	54 (36%)	98 (64%)	152 (100%)
	Partial	85 (50%)	85 (50%)	170 (100%)
	Unclassified	2 (33%)	4 (67%)	6 (100%)
	Total	141 (43%)	187 (57%)	328 (100%)

***$P < 0.001$ between the intellectually normal and the intellectually disabled groups.

Table 6 Overall improvement rating: generalized epilepsies.

	Epilepsies	Improved	Unimproved	Total
Intellectually normal	PGE	43 (91%)***	4 (9%)	47 (100%)
	West syndrome	1 (100%)	0	1 (100%)
	LGS	3 (50%)	3 (50%)	6 (100%)
	Other SGE	5 (56%)	4 (44%)	9 (100%)
	Unclassified GE	3 (75%)	1 (25%)	4 (100%)
	Total	55 (82%)***	12 (18%)	67 (100%)
Intellectually disabled	PGE	11 (58%)	8 (42%)	19 (100%)
	West syndrome	7 (33%)	14 (67%)	21 (100%)
	LGS	7 (21%)	26 (79%)	33 (100%)
	Other SGE	26 (40%)	39 (60%)	65 (100%)
	Unclassified GE	3 (21%)	11 (79%)	14 (100%)
	Total	54 (36%)	98 (64%)	152 (100%)

PGE = primary generalized epilepsy; LGS = Lennox-Gastaut syndrome; SGE = secondarily generalized epilepsy; GE = generalized epilepsy.
*** $P < 0.001$ between the intellectually normal and the intellectually disabled groups.

Table 7 Overall improvement rating: partial epilepsies.

	Epilepsies	Improved	Unimproved	Total
Intellectually normal	TLE	36 (65%)	19 (35%)	55 (100%)
	Non-TLE	177 (79%)***	47 (21%)	224 (100%)
	Others	43 (83%)***	9 (17%)	52 (100%)
	Total	256 (77%)***	75 (23%)	331 (100%)
Intellectually disabled	TLE	12 (50%)	12 (50%)	24 (100%)
	Non-TLE	61 (51%)	59 (49%)	120 (100%)
	Others	12 (46%)	14 (54%)	26 (100%)
	Total	85 (50%)	85 (50%)	170 (100%)

TLE = temporal lobe epilepsy.
*** $P < 0.001$ between the intellectually normal and the intellectually disabled groups.

Table 8 shows the relationship between improvement rates and age of epilepsy onset. In any age group, the improvement rating was greater ($P < 0.001$) in the intellectually normal group (78%) than in the intellectually disabled group (43%).

Safety

Adverse effects were experienced by 21% of the intellectually normal patients, and by 28% of the intellectually disabled patients (see Table 9). The

Table 8 Overall improvement rating.

Age of onset (years)	Improved (reduction ≥50% of seizure frequency)		
	Intellectually normal	Intellectually disabled	Total
<1	12/14 (86%)	1/6 (17%)	13/20 (65%)
1–5	60/83 (72%)	52/120 (43%)	112/203 (55%)
6–10	140/170 (82%)	46/100 (46%)	186/270 (69%)
11–15	102/134 (76%)	42/102 (41%)	144/236 (61%)
Total	314/401 (78%)***	141/328 (43%)	455/729 (62%)

*** $P < 0.001$ between the intellectually normal and the intellectually disabled groups.

Table 9 Adverse effects of zonisamide.

	Patients experiencing adverse effects (%)	Patients experiencing no adverse effect (%)	Total
Intellectually normal	86 (21%)	332 (79%)	418 (100%)
Intellectually disabled	96 (28%)	245 (72%)	341 (100%)
Total	182 (24%)	577 (76%)	759 (100%)

Table 10 Adverse effects of zonisamide: monotherapy vs. combination therapy.

Age of onset (years)	Zonisamide monotherapy	Zonisamide combination therapy	Total
<1	2/14 (14%)	3/6 (50%)	5/20 (25%)
1–5	13/71 (18%)	34/142 (24%)	47/213 (22%)
6–10	15/112 (13%)	48/170 (28%)	63/282 (22%)
11–15	14/70 (20%)	53/174 (30%)	67/244 (27%)
Total	44/267 (16%)***	138/492 (28%)	182/759 (24%)

*** $P < 0.001$ (monotherapy vs. combination therapy).

incidence of adverse events was slightly higher in the intellectually disabled group, but there is no significant difference between these groups. In total, 24% of the 759 patients in this study experienced some adverse effects.

In Table 10, a comparison of adverse events observed with zonisamide monotherapy (16%) versus combined therapy (28%) demonstrates that fewer adverse effects were observed in the monotherapy group ($P < 0.001$). There were no significant differences in adverse events between age groups, except that in the combined therapy group of patients under 1 year of age, the incidence of adverse effects was increased (50%).

Discussion and conclusions

Before the commercial licensing of zonisamide in Japan, a placebo-controlled study in children showed that the percentage of patients with a 50% or higher decrease in seizure frequency was 50.0% and 43.8%, by zonisamide and valproate, respectively.[4] This study was done by 8 weeks of observation. Our study of long-term observation showed that the overall improvement rate was 62%, which was slightly higher than the placebo-controlled study results. The patients evaluated before launch had more frequent seizures—more than 4 times a month. After commercial licensing, zonisamide was also administered to many patients having infrequent seizures. The difference of the efficacy of zonisamide before and after the licensure is likely due to the background seizure frequency of the patients. In intellectually normal children, the overall improvement rate was thought to be quite high (78%); furthermore, patients with generalized epilepsy in the intellectually normal group showed 82% improvement.

It has generally been thought that zonisamide is more efficacious for partial epilepsy than generalized epilepsy.[5] It was reported that patients who had some temporal lobe abnormalities verified by EEG, computed tomography, or positron emission tomography responded well to zonisamide.[6] In that report, zonisamide was effective for partial epilepsy as well as generalized epilepsy with temporal lobe lesions. In the present study, however, zonisamide demonstrates efficacy for generalized epilepsy equal to or greater than its efficacy for partial epilepsy. The patients with generalized epilepsy who responded well to zonisamide might have had some temporal lobe abnormalities.

In comparing the efficacy of zonisamide for intellectually normal and disabled patients, zonisamide was more effective for intellectually normal patients. In general, intellectually disabled children have various kinds of central nervous system disturbances. These disturbances relate to the severity of seizures in epileptic patients with intellectual disability. However, even for intellectually disabled patients, zonisamide had good efficacy for seizure control. Zonisamide can be recommended for the treatment of both partial and generalized seizures in intellectually disabled children with epilepsy.

Treatment-emergent adverse effects were observed to have almost the same incidence in the normal intelligence and the intellectually disabled

groups. Zonisamide monotherapy demonstrated less frequent adverse effects than zonisamide combined therapy. Combined antiepilepsy therapy often induces adverse effects more frequently in any antiepilepsy drug, not only zonisamide.[7]

In the treatment of epileptic children with zonisamide, anhidrosis accompanied by elevated body temperature has been reported, especially during summer.[8,9] Moreover, Shimizu et al. reported a case of an intellectually disabled 2-year-old boy demonstrating a heat stroke-like episode associated with zonisamide treatment.[10] This patient showed hyperpyrexia and oligohidrosis. In the present study, cases with anhidrosis were not reported by the doctors. However, anhidrosis or oligohidrosis is often difficult to observe, especially by intellectually disabled patients. Hyperpyrexia and oligohidrosis should be carefully monitored during zonisamide treatment.

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Clinical Research

Dynamic Cortical Activity during Spasms in Three Patients with West Syndrome: A Multichannel Near-infrared Spectroscopic Topography Study

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Summary: *Purpose:* To investigate spatial and temporal cortical activity during clusters of naturally occurring epileptic spasms in patients with West syndrome (WS) by using multichannel near-infrared spectroscopy (mNIRS).

Methods: Conventional magnetic resonance imaging (MRI) and interictal and ictal single-photon emission computed tomography (SPECT) were carried out in three patients with WS. Thereafter, cortical hemodynamics during naturally occurring epileptic spasms were measured by mNIRS with simultaneous video/electroencephalographic (EEG) monitoring.

Results: Ictal SPECT revealed multiple hyperperfused areas within the cortex. With the use of mNIRS, an increase in regional cerebral blood volume (CBV) was observed in these areas, which is representative of cortical activation. The increase in CBV was accompanied by an increase in the concentrations of both oxy- and deoxyhemoglobin. The following heterogeneous regional changes in CBV during ictus were observed: (a) transient increases that were synchronized with spasms; (b) a gradual increase during an ictal event that fluctuated in synchrony with spasms; and (c) a combination of transient and gradual increases. An increase in regional CBV occurred in multiple areas that were activated either simultaneously or sequentially during an ictal event. Topographic changes in CBV were closely correlated with the phenotype of the spasm.

Conclusions: During ictal events, multiple cortical areas were activated simultaneously or sequentially. The pattern of cortical activation closely affected the phenotype of the spasm, which suggested that the cortex was involved in the generation of spasms. **Key Words:** West syndrome—Infantile spasm—Near-infrared spectroscopy.

West syndrome (WS) is an age-dependent epileptic syndrome that occurs during infancy. WS is characterized by clustering epileptic spasms, electroencephalographic (EEG) hypsarrhythmia, and arrested psychomotor development (1,2). Although the pathophysiology of WS remains unknown, subcortical structures such as the lentiform nuclei and brainstem are thought to be involved (3,4). In addition, magnetic resonance imaging (MRI) and functional neuroimaging have revealed that a considerable number of children with WS possess focal cortical abnormalities (5–7). Surgical resection of the affected area can eliminate the spasms (8), suggesting (at least in such patients) that the abnormal cortex may play a crucial role in the pathogenesis of spasms in patients with WS.

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Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have suggested that abnormal cortical areas are hyperperfused during ictal periods, implying that these areas of the cortex are activated during a cluster of spasms (9,10). However, because of the insufficient time resolution of PET and SPECT for rapidly progressing and repeatedly occurring spasms, it is impossible to discriminate between the cerebral perfusion changes during spasms and those at intervals of spasms, and postictal changes might contaminate the results. A different neuroimaging modality with superior time resolution is thus required to delineate the time course of cortical activity during the progression of the ictal events of WS.

Recently, multichannel near-infrared spectroscopy (mNIRS) has been used to investigate higher brain function because changes in regional cerebral blood volume (CBV), which can be measured by using mNIRS, are correlated closely with the topology of cortical activity (11,12). The relatively good time resolution of mNIRS

TABLE 1. *Clinical characteristics of patients*

	Case 1	Case 2	Case 3
Sex	female	female	male
Gestational age at birth (weeks)	35	37	40
Age (months)			
at onset	3	7	5
at examination	3	8	6
Seizure type	symmetrical spasms followed by asymmetrical spasms (L>R)	asymmetrical spasms (L>R) followed by symmetrical spasms	partial seizure followed by symmetrical spasms

overcomes the aforementioned limitation of PET and SPECT, and therefore it might be a useful method for observing spatial and temporal changes in cortical activity during ictus in patients with epilepsy (13). Here, we used mNIRS to assess the involvement of the cerebral cortex in ictal events in patients with WS.

METHODS

Patients

We studied three patients with WS. The clinical characteristics of the patients are summarized in Table 1.

Case 1

A girl (the first of twins) was born after 35 weeks of gestation; pregnancy and delivery were uncomplicated. The mother had Crohn's disease, but no family history of epilepsy was known. The first seizure occurred at age 3 months. During the ictal period, the girl exhibited brief extension of the upper and lower limbs, which was associated with upward rolling of the eyes. Initially, the spasms were symmetrical but became asymmetrical toward the end of a cluster in association with weak extension of the left upper limbs. An interictal EEG recording revealed hypsarrhythmia. The results of conventional MRI, retinal observation, and blood analyses (including lactate and amino acid analyses) were normal. Despite treatment for 2 weeks with vitamin B_6 and valproate (VPA), the infantile spasms remained. Although subsequent treatment with adrenocorticotropic hormone (ACTH) eliminated the spasms and the hypsarrhythmia, a brief tonic seizure without clustering occurred 1 month later.

Case 2

A girl was born after 37 weeks of gestation; pregnancy was uncomplicated. The first seizure was noted at age 7 months. During the ictal period, the girl exhibited brief spasms in clusters. Hypsarrhythmia was noted in an EEG recording. Vitamin B_6 and VPA were found to be ineffective, and she was referred to our hospital at age 8 months. MRI revealed a small lesion of unknown etiology at the posterior lateral aspect of the left lateral ventricle, whereas retinal observation and blood analyses were normal. Then ACTH treatment was carried out, and the spasms ceased, with an EEG improvement.

Case 3

A boy was born after 40 weeks of gestation; pregnancy was uncomplicated. The first seizure was noted at age 5 months. During the ictal period, he exhibited brief extension of the upper limbs with head-nodding. At age 6 months, he could no longer control the movements of his head, and he was referred to our hospital. Hypsarrhythmia was noted in an interictal EEG. The MRI, retinal observation, and metabolic parameters were normal. ACTH treatment was carried out after vitamin B_6 and VPA were found to be ineffective. After the ACTH treatment, the hypsarrhythmia disappeared, and the spasms ceased.

Methods

Within 2 weeks of admission to the Tohoku University Hospital and before the initiation of ACTH therapy, interictal and ictal SPECT and ictal mNIRS with video-EEG monitoring were carried out in each of the three aforementioned patients. We received informed consent from the parents of the patients for the following examinations.

For ictal SPECT, as soon as the attending pediatrician noticed the occurrence of spasms, [^{99m}Tc]-ethyl cysteinate dimer (ECD) was injected intravenously. The fixation of [^{99m}Tc]-ECD within the brain after injection required several minutes (14). Because multiple spasms occurred during this period, the SPECT images obtained represented mixed cerebral blood flow (CBF) during the multiple spasms. For the interictal SPECT study, [^{99m}Tc]-ECD was injected while the patient was conscious. Both ictal and interictal SPECT images were acquired at 30 min after the injection of [^{99m}Tc]-ECD by using a MULTI-SPECT 3 (Siemens, Gerfahldt, Germany). The patient was sedated with diazepam (DZP) during the scanning. The method that we used to construct a tomogram of CBF has been described elsewhere (10).

NIRS is based on the fact that near-infrared light can propagate through biologic material with scattering, and the absorption during propagation is inversely related to hemoglobin (Hb) concentrations in tissues (15). By using two specific wavelengths of near-infrared light (see later), the relative changes in oxy- and deoxy-Hb concentrations can be calculated separately (11). The summation of oxy- and deoxy-Hb concentrations gives the change in total Hb (total-Hb) concentrations, which is representative of changes in CBV (16).

In the present study, a mNIRS system (ETG 100; Hitachi Medical Corporation, Tokyo, Japan) was used. After the placement of selected scalp electrodes for digital EEG monitoring (EEG2100; Nihon Kohden, Tokyo, Japan), two optode arrays for mNIRS were fixed to the scalp by using a tubular net bandage and surgical tape. Based on the SPECT results, the arrays were placed bilaterally symmetrically with the center of each array located approximately

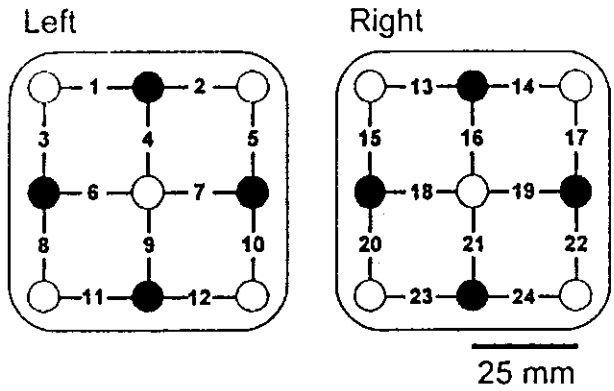

FIG. 1. Configuration of optode arrays. Each array consisted of alternately arranged light-emitting (*solid circles*) and light-detecting (*open circles*) optodes. Absorption of near-infrared light (780 and 840 nm) was measured among adjoining emitting and detecting optodes, thereby producing 12 recording channels per hemisphere (channels 1–12, right hemisphere; 13–24, left hemisphere). The gray area illustrates the extent of the topographic image that was constructed by using spatial spline interpolation across the recording channels.

over the frontal or central area (10-20 system). The attachment for the arrays was fabricated specifically for infants to minimize discomfort (Hitachi Medical Corporation). Each array comprised four light-emitting and five light-detecting optodes that were arranged alternately with a spacing of 25 mm (Fig. 1). The absorption of backscattered light at wavelengths of 780 and 840 nm was measured between adjoining optodes, thereby producing 12 measurement channels per hemisphere. The sampling interval was 0.5 s. For off-line analysis, baseline values of oxy-, deoxy-, and total-Hb were aligned to the period that immediately preceded the ictal period (unless otherwise stated). Topographic images of the changes in regional CBV were constructed by using spline interpolation. The spatial resolution of the images was limited by interoptode distance.

RESULTS

Figure 2A shows the results of the interictal (Fig. 2A, a–d) and ictal SPECT (Fig. 2A, e–h) for case 1. During ictus, a prominent increase in regional CBF was noted in the right anterior frontal lobe (arrow in Fig. 2A, e), which was surrounded by a relatively hypoperfused area (arrowhead). The contralateral cortex also was hyperperfused with a predominant increase in regional CBF in the left frontal area (Fig. 2A, e and f). In addition, an increase in regional CBF during ictus was observed in subcortical structures such as the bilateral lentiform nuclei, cerebellum, and brainstem (Fig. 2A, g and h).

In case 1, 19 spasms were recorded during mNIRS monitoring. Figure 2B shows representative topographic maps of the spatial changes in total-Hb concentrations (which reflect regional changes in CBV) in the bilateral frontal area during spasms. A focal increase in CBV occurred in the right hemisphere, whereas a simultaneous decrease in CBV occurred in the adjacent area. In the left hemisphere, CBV increased in a relatively diffuse manner. Figure 2C, a and b, illustrate the time course of changes in total-, oxy-, and deoxy-Hb concentrations during a cluster of spasms in the right hemisphere at the channels indicated by (a) and (b) in Figure 2B. In Figure 2C, a, the increase in regional CBV was transient, and the increase was synchronized with the spasm (arrowheads). In addition, a peripheral decrease in CBV (Fig. 2C, b) also was synchronized with the spasm. The first 11 spasms were intensely tonic and symmetrical (solid arrowheads in Fig. 2C); thereafter, the spasms became weak and asymmetrical (open arrowheads), and the spasms were associated with extension of the left arm. Intense spasms tended to be correlated with high total-Hb concentrations.

Figure 2D shows representative ictal EEG recordings during a spasm. A focal spike in the right hemisphere preceded each spasm. After this, fast waves occurred with right-hemisphere dominance; these waves were superimposed with high-voltage slow waves and were followed by desynchronization. The dotted line in Figure 2D indicates the onset of the spasm as determined by surface electromyography (EMG).

Figure 3A shows the results of interictal (Fig. 3A, a–d) and ictal (Fig. 3A, e–h) SPECT for case 2. During ictus, a bilateral increase in regional CBF was observed in the parietooccipital cortex, along with an increase in regional CBF in the bilateral lentiform nuclei. Consequently, for case 2, the optode arrays for mNIRS were placed symmetrically bilaterally, with the center of each array located over the central area (10-20 system), as illustrated in the insets in Figure 3B.

Figure 3B shows topographic maps of the regional CBV changes around the right and left central areas during a spasm for case 2 at the time point indicated by an asterisk in Figure 3C. A focal increase in CBV was seen in the posterior region of the right topographic map. A slight increase in CBV was observed also in the left homologous site. Figure 3C shows the time course of changes in total-, oxy-, and deoxy-Hb concentrations during a cluster of spasms in the right (R) and left (L) hemispheres at the same points in the corresponding hemispheres (solid circles in Fig. 3B). The spasms gradually became more intense during the progression of a cluster, and continuous recording in the right hemisphere became impossible after the eighth spasm, owing to the development of intense motion (broken line in Fig. 3C). Initially, the transient increase in CBV was synchronized with spasms in the right hemisphere; thereafter, a periodic increase in CBV in the left hemisphere gradually became obvious.

Figure 3D, a–c, shows an ictal EEG and surface EMG recordings that were made at the bilateral deltoid muscle;