Influence of Concomitant Antiepileptic Drugs on Plasma Lamotrigine Concentration in Adult Japanese Epilepsy Patients

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Lamotrigine (LTG) is an antiepileptic drug (AED) that was approved in Japan in 2008. We evaluated the influence of AEDs that induce hepatic enzymes (including phenytoin (PHT), phenobarbital (PB), carbamazepine (CBZ)), valproic acid (VPA), and various combinations of these drugs, on plasma LTG concentration in adult Japanese epilepsy patients. A total of 621 patients (mean age 34.4±11.8 years) were evaluated retrospectively. We calculated the concentration to dose ratio (CD ratio) for LTG with different AED regimens, and employed multiple regression analysis to determine factors influencing the LTG concentration. There was a linear correlation between the dose and concentration of LTG in patients treated with LTG (group I), LTG+VPA (group II), LTG+inducers (group III), or LTG+VPA+inducers (group IV). The mean CD ratio of patients on LTG monotherapy was 1.43±0.4 (μg/mL)/(mg/kg). When LTG was combined with VPA, the CD ratio increased about 2.2-fold, but there was no significant correlation between the CD ratio and VPA concentration. The mean CD ratios calculated in patients receiving LTG+PHT, LTG+PB, and LTG+CBZ were 0.56, 0.84, and 0.91, respectively. Addition of PHT significantly reduced the CD ratio in a concentration-dependent manner, in comparison with PB and CBZ (p<0.005 and p<0.001, respectively). Stepwise multiple regression analysis showed that the coefficient of determination of groups I, II, III, and IV were 0.94, 0.94, 0.90, and 0.91, respectively. In the clinical setting, these findings can help to estimate LTG concentrations and predict the inducing or inhibiting effects of concomitant AEDs.

Key words lamotrigine; drug interaction; therapeutic drug monitoring; concentration–dose ratio

Lamotrigine (LTG) is widely used in the treatment of epilepsy and bipolar disorder. In Japan, LTG is a novel antiepileptic drug (AED) that was approved in 2008 as an add-on treatment for partial, secondarily generalized, and tonic-clonic seizures in patients with refractory epilepsy. LTG is also approved for generalized seizures associated with Lennox–Gastaut syndrome.

Therapeutic drug monitoring (TDM) is usually recommended to optimize AED therapy, but the usefulness of TDM for LTG is controversial. Several reports have suggested that there is no relationship between the clinical response or side effects and the plasma concentration of LTG.1–3 In contrast, Johannessen and Tomson9 reported that LTG was likely to be useful for treatment with LTG in many clinical settings. Hirsch et al.9 also reported that the incidence of side effects caused by LTG increased along with elevation of its concentration. According to this report, full seizure control could be obtained for a 6-month period by maintaining plasma LTG concentrations above 20 μg/mL. However, there was a significant increase (p<0.001) of side effects as the LTG concentration increased, with 14% of patients showing side effects at concentrations of 5.0 to 10 μg/mL, 24% at 10 to 15 μg/mL, and 59% above 20 μg/mL. In Australia, Morris et al.6 reported that requests for TDM related to LTG increased 2.9-fold from 1996 to 2003. Thus, it may be important for individualized therapy to monitor the plasma concentration profile of LTG.

LTG is mainly metabolized to an inactive metabolite (N-2 glucuronide) by three uridine-5′-diphosphate-glucuronosyltransferases (UGT) 1A4,7 2B7,8 and 1A1.9 The plasma concentration of LTG is known to be influenced by other AEDs. Especially, AEDs that induce the hepatic drug metabolizing enzymes (inducers), such as phenytoin (PHT), carbamazepine (CBZ), and phenobarbital (PB), reduce the plasma LTG concentration, while concomitant administration of valproic acid (VPA) increases the LTG concentration.6,10–16 Accordingly, different dosages of LTG are required when it is used concomitantly with VPA or with inducers. However, there have been few reports of blood LTG concentration data in epilepsy patients receiving LTG monotherapy. Also, there is limited information about the LTG concentration profile in patients treated with a combination of LTG plus multiple inducers or LTG plus VPA and one or more inducers. Moreover, little is known about ethnic differences in the pharmacokinetics of LTG. Hussein and Posner17 reported that clearance of LTG was approximately 29% lower in Asians than in Caucasians, but Asians accounted for only 5 of 163 patients in their study. In addition, Grasela et al.18 reported that LTG clearance was reduced by 25% in non-Caucasians, but the ethnicity of their non-Caucasian subjects was not clarified. Thus, it is important to verify whether the pharmacokinetics of LTG in Japanese patients are equivalent to those in Caucasians.

At the National Epilepsy Center (Shizuoka, Japan), TDM has been performed for LTG since 2009. The aim of this study was to investigate the plasma LTG concentration profile under different circumstances and identify influencing factors such as age, gender, and combination of inducers, VPA, and other AEDs in Japanese patients with epilepsy.

MATERIALS AND METHODS

Subjects The study protocol was approved by the ethical committee of National Epilepsy Center (Shizuoka, Japan).

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This study enrolled 621 adult patients (307 men and 314 women; mean age 34.4±11.8 [16–76] years) with epilepsy who were treated with LTG (Lamictal, tablets, Glaxo SmithKline K.K., Japan) for epilepsy at our hospital between January 2009 and December 2010. In addition, 134 adult patients (73 men and 61 women with a mean age of 29.6±12.5 years) treated with LTG between January 2011 and April 2011 were recruited for comparison of the estimated and measured LTG concentrations. Most of the patients were unresponsive to standard AED regimens. Patients using primidone or oral contraceptives, and suffering from serious hepatic or renal dysfunction were excluded. In this study, information about the patient’s age, gender, body weight, LTG dose and concentration, and concomitant AEDs were retrospectively obtained from the clinical records.

**Blood Sampling and LTG Analysis** Blood samples were obtained from patients at 2 to 5 h after dose of LTG, and were centrifuged at 3000 rpm. The plasma LTG concentration was analyzed by using the HPLC method with a reversed-phase column. Other AEDs were analyzed by a latex immunagglutination assay. The steady-state LTG concentration was obtained after LTG and other AEDs had been maintained on the same dosing schedule for at least 28 d. When two or more blood samples were obtained from the same patient during the study, the average value in samples at the highest maintenance dose of LTG was adopted. Data on the body weight and other AEDs were treated in the same manner.

**Statistical Methods** To analyze the relationship between concomitant AEDs and the concentration to dose ratio (CD ratio (μg/mL per mg/kg)) for LTG, patients were classified according to the concomitant AEDs. Analysis of variance and the Games–Howell or Dunnett test were employed. Correlations were assessed by Pearson’s or Spearman’s correlation coefficient analysis after testing for normality. Factors influencing the LTG concentration were identified by using a multiple regression model. Results are expressed as the mean±standard deviation. The level of significance was set at p<0.05. Statistical analyses were conducted with IBM SPSS Statistics Ver 19 (IBM Japan).

**RESULTS**

**Patient Characteristics** The patients receiving LTG were classified into four groups according to their concomitant AEDs: group I received LTG alone or with non-inducers such as clobazam, zonisamide, clonazepam, topiramate, levetiracetam, gabapentin, or ethosuximide, group II received LTG+VPA or LTG+VPA+non-inducers, group III received LTG+inducers such as PHT, PB, or CBZ, and group IV received LTG+VPA+inducers (Table 1). The age, gender, and LTG dose, concentration, and CD ratio were significantly different among the four groups.

**Correlation between LTG Concentration and Dose** Figure 1 shows the correlation between the dose and concentration of LTG in the four groups. There was a strong linear correlation between the dose of LTG and its concentration in all four groups, but there was a large variation of the concentration in patients receiving high-dose LTG. The slope was markedly increased by concomitant use of VPA until it was almost twice as steep. In contrast, combinations of inducers (groups III and IV) resulted in a marked decrease of the slope compared with groups I and II.

**Influence of VPA Plus Non-inducers on the CD Ratio of LTG** The mean CD ratios for LTG monotherapy and combination therapy with non-inducers were 1.43±0.4 and 1.31±0.5, respectively, and no significant difference was found. In patients receiving LTG and VPA, the CD ratio obtained from patients with and without non-inducers was 3.08±1.1 and 3.12±0.9, respectively, and there was also no significant difference. Hence, we considered that non-inducers had an insignificant effect on plasma LTG concentration.

**Comparison of CD Ratios in Patients Receiving Inducers** Figure 2 shows a comparison of the CD ratios for LTG in patients receiving different inducers. The mean CD ratios in patients receiving PHT, PB, and CBZ were 0.56, 0.84, and 0.91, respectively. PHT significantly reduced the CD ratio of LTG in comparison with PB and CBZ (p<0.005 and 0.001, respectively). The CD ratios for LTG in patients receiving PHT+PB and PHT+CBZ were 0.58 and 0.64, respectively, and these show a significant decrease in comparison with CBZ.

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<th>Table 1. Characteristics of the Patients</th>
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Group I; 49 patient with LTG monotherapy and 47 patients with LTG+non-inducers. Group II; 62 patient with LTG+VPA and 37 patients with LTG+VPA+non-inducers. Group III; 197 patient with LTG+inducers and 81 patients with LTG+inducers+non-inducers. Group IV; 112 patient with LTG+VPA+inducers and 36 patients with LTG+VPA+inducers+non-inducers. S.D.; standard deviation; BMI; body-mass index; BSA; body surface area; CD ratio, concentration to dose ratio. Significance was determined by ANOVA or χ² test. Games–Howell post-hoc test; *p<0.05 versus Group I, †p<0.005 versus Group II, ‡p<0.001 versus Group III, §p<0.001 versus Group I, p<0.001 versus Group II.
Fig. 1. Correlation between the Dose and Concentration of LTG
The dose and concentration data were normally distributed in all groups. $R^2$, the coefficient of determination; $r^2$, the squared correlation coefficient.

Fig. 2. Box Plots for the CD Ratio of LTG in Patients with Different Inducers (Group III)
The bottom and top of the box show the 25th and 75th percentiles, respectively. The horizontal line in each box indicates the median. Open circles are outliers. Significance was determined by ANOVA ($p<0.001$). Results of the Games–Howell post-hoc test are shown in the above table. * Dunnett’s test; LTG monotherapy versus groups 1–7; $p=0.001$. 
Figure 3 shows CD ratios for LTG in patients receiving VPA and inducers. The mean CD ratios were 1.45 (VPA+PHT), 1.95 (VPA+PB), 1.95 (VPA+CBZ), 1.10 (VPA+PHT+PB), 1.22 (VPA+PHT+CBZ), and 2.01 (VPA+PB+CBZ), suggesting that patients receiving LTG plus VPA+PHT had lower ratios than those using VPA+PB or VPA+CBZ (p<0.05), and similar findings were seen in the patients receiving multiple inducers. These results were similar to those shown in Fig. 2.

Correlation between the CD Ratio for LTG and Plasma Concentrations of Combined AEDs

Figure 4 shows the correlations between the CD ratio for LTG and the plasma concentrations of VPA and PHT. There was a significant negative correlation between the CD ratio for LTG and the concentration of PHT (r²=0.23, p<0.001). In particular, when the PHT concentration was less than 10 μg/mL, the mean CD ratio increased from 0.50 to 0.82. Meanwhile, the concentration of VPA showed no significant correlation with that of LTG. Also, there were no correlation between the CD and the concentration of PB and CBZ (r²=0.053 (p=0.30) and r²=0.012 (p=0.32), respectively).

Results of Multiple Regression Analysis

Table 2 shows factors influencing the LTG concentration according to multiple regression analysis using the stepwise selection procedure. Multiple regression analysis showed that the coefficient of determination (R²) for both groups I and II was 0.94, and the variable of gender was eliminated by stepwise selection. In group III, R² was 0.90 and the variables eliminated were age, as well as the concomitant use of PHT, PHT+PB, PHT+CBZ, and PHT+PB+CBZ. In group IV, the variables eliminated (in the following order) were age, gender, and concomitant use of VPA+PHT+PB and VPA+PHT+CBZ, resulting in a model with five predictors and an R² value of 0.91. Thus, more than 90% of the LTG concentration could be explained by factors such as the age, gender, LTG dose, and concomitant administration of PHT, PB, CBZ, VPA, or a combination of these drugs (Table 2). For example, when a 30-year-old woman weighing 50 kg received 200 mg of LTG combined with CBZ, the steady-state concentration of LTG could be estimated as follows.

\[
\text{LTG concentration (μg/mL)} = -0.35 (\text{gender}) + 0.64 \times 4 (\text{LTG; 4 mg/kg}) + 1.2 (\text{CBZ = 1}) = 3.4 \text{ μg/mL}
\]

In Table 2, the partial regression coefficients are similar to data on the CD ratios for LTG shown in Figs. 2 and 3. PHT most strongly reduced the LTG concentration among the inducers.

Comparison of Estimated and Measured LTG Concentrations

The LTG concentration was measured in 134 adult patients (group I; n=19, group II; n=15, group III; n=67, and group IV; n=33), while estimated concentrations were calculated using the multiple regression model. Figure 5 shows the relation between estimated and measured LTG concentrations. There was a strong positive correlation between the two parameters (r²=0.72, p<0.001). In 37.3% (50/134) of the patients, the difference between the measured and estimated LTG concentrations (residual) was >1 μg/mL, and the mean absolute residual was 1.15 μg/mL.
DISCUSSION

Since LTG was first approved in 1991, several reports about the influence of concomitant AEDs on the LTG concentration have been published. In Japan, however, little information about TDM for LTG has been obtained. This is the first report about the influence of concomitant AEDs on the LTG concentration, and the first study to establish estimated values and parameters based on measurement of LTG in Japanese patients with epilepsy.

LTG is mainly metabolized by UGT1A4 and 2B7, and its bioavailability is 95% or higher. In recent years, it has been reported that the LTG concentration is influenced by UGT1A4 (142T>G) and 2B7 (−161C>T) polymorphism. Gulcebi et al. found that the mean apparent clearance of LTG in Turkish patients with UGT1A4 (142T>G) polymorphism receiving LTG monotherapy was 1.9-fold higher than that in wild-type patients. Thus, this polymorphism causes an increase of glucuronidation activity.

On the other hand, the frequency of UGT2B7 (−161C>T) polymorphism, which is linked with −161C>T, was reported to be 48.9–53.7% in Caucasians and only 24.4–29.3% in Japanese. Four studies about the CD ratio of LTG in European patients have been published. Among them, Sánchez et al. reported a significant association between the CD ratio of LTG and UGT2B7 (−161C>T) polymorphism. They found a CD ratio of 1.65±1.5 in patients with the CC genotype (wild-type), which was higher than the ratios of 1.24±1.2 and 1.20±1.0 in patients with the CT and TT genotypes, respectively. In contrast, a higher CD ratio was observed in adult patients with the TT genotype than in those with the CC or CT genotype among patients receiving LTG monotherapy (1.98±0.5 vs. 1.10±0.3, p<0.001), but only 3 patients had the TT genotype.

In Japanese patients on LTG monotherapy (49 out of 96 patients in group I), the CD ratio was 3.10±1.0 in Japanese patients...
receiving LTG+VPA (group II), which was lower than the ratios of 3.84±1.19 and 4.04±0.92 in Spanish patients, 3.4±1.7 in Italian patients,10,19 or 3.66±1.7 in German patients.19

Chung et al.20 reported that the area under the concentration vs. time curve (AUC) of VPA became larger as the number of UGT2B7 (−161C>T) alleles increased in healthy volunteers receiving lorazepam, which is a competitive inhibitor of UGT2B7. Lorazepam, VPA and LTG are substrates of UGT2B7. An in vitro study by Rowland et al.8 showed that VPA inhibits UGT2B7-catalyzed glucuronidation of LTG. Thus, further in vivo studies will be necessary to evaluate the association between UGT2B7 polymorphism and inhibition of LTG metabolism by VPA.

Our data also showed that VPA strongly inhibited LTG metabolism. However, there was no significant correlation between the CD ratio of LTG and the VPA concentration (Fig. 4). Gidal et al.27 reported that VPA inhibits the clearance of LTG at a low dose (125 mg/d) and a very low concentration (6.5 μg/mL). In our study, only 2 out of 62 patients (3.2%) were on a low dose of VPA (less than 200 mg/d). This small number of patients with low-dose VPA therapy may have contributed to the lack of a correlation between the VPA level and the CD ratio for LTG.

In general, PHT, PB, and CBZ induce cytochrome P450 (CYP) and UGT enzymes. Induction of LTG metabolism by all of PHT, PB, and CBZ was observed in our study, but PHT was the strongest inducer. According to a study on the pharmacokinetics of VPA, its clearance was increased about 1.5-fold by concomitant administration of PHT in comparison with VPA monotherapy, which was higher than seen with concomitant administration of PB or CBZ.28 Thus, LTG metabolism is induced in the same manner as VPA. May et al.10 reported no statistically significant difference between the CD ratio for LTG and the PHT concentration. In contrast, our study revealed that PHT decreased the CD ratio for LTG in a concentration-dependent manner. In particular, when the PHT concentration was low therapeutic range (<10 μg/mL), the mean CD ratio increased about 1.6-fold. Our findings indicate that PHT induces UGT enzymes (in a concentration-dependent manner) more strongly than PB or CBZ.

A number of studies have stratified subjects into those with or without inducers or by the number of inducers (such as single or multiple inducers).6,12,13,17,18 We classified the subjects by different inducer combinations and compared the CD ratios for LTG in these groups (Figs. 2, 3). As a result, the combination of LTG with PHT led to a marked decrease of the CD ratio, but PHT did not have a synergistic effect in combination with PB and/or CBZ. This suggests that induction of UGT enzymes might have reached a plateau. Moreover, PB and CBZ induce CYP enzymes, resulting in a decrease of PHT concentration that may contribute to alleviating the induction of UGT enzymes by PHT.

Age and gender were significantly different among the four groups (Table 1). Accordingly, we calculated partial regression coefficients for factors influencing the LTG concentrations by multiple regression analysis (Table 2). Our findings allowed us to estimate the LTG concentration in Japanese patients with epilepsy by applying coefficients for age, gender, LTG dose, and the AED regimens to a multiple regression model. In addition, the $r^2$ value for the relation between the measured and estimated LTG concentrations was 0.72 ($p<0.001$) in our model, which was equal that reported for a multiple regression model ($r^2=0.72$) and better than that for a population pharmacokinetic model ($r^2=0.62$) in Caucasian patients (Fig. 5).

In the clinical setting, patients who have refractory epilepsy are generally treated with multiple AEDs and changes of regimen, such as addition or discontinuation of drugs, are frequent. We calculated the estimated value and the change ratio of the LTG concentration using a multiple regression model. When AEDs were changed in a patient, the LTG concentration could be predicted from Table 2. For example, when PHT is added in a patient receiving LTG monotherapy, the LTG concentration is expected to decrease to about 50%, while addition of VPA can compensate for the effect of PHT.

This study had several limitations. Among the patients receiving inducers, only 15 patients were administrated primidone. Accordingly, these patients were excluded because it was difficult to stratify the group using primidone. In addition, there were only 8 patients receiving the VPA+PB+CBZ combination. Furthermore, trough LTG concentrations were not measured. Despite the lack of trough data, there was still a strong linear relationship between the dose and concentration of LTG (Fig. 1), as well as significant differences among the combination of AEDs (Figs. 2, 3).

In conclusion, our study showed that drug interactions between LTG and PHT, PB, CBZ, VPA, or combinations of these drugs in Japanese patients with epilepsy could be quantified by multiple regression analysis. Estimation of the LTG concentration is important when adding or discontinuing PHT, PB, CBZ, and VPA. Our data can be employed to estimate LTG concentrations and the inducing or inhibiting effects of various AEDs in the clinical setting.

Further studies will be necessary to evaluate the influence of UGT1A4 and 2B7 polymorphism on the clinical efficacy and side effects of LTG.

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