THE EFFECT OF SERUM LEVETIRACETAM CONCENTRATIONS ON THERAPEUTIC RESPONSE AND IL1-BETA CONCENTRATION IN PATIENTS WITH EPILEPSY

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Abstract

Objective: Assessment of the relevance between serum drug concentration to its therapeutic response is a valid monitoring strategy for the clinical efficacy of antiepileptic drugs (AEDs). Levetiracetam (LEV) is a broad spectrum AED with a possible anti-inflammatory effect. We aimed to determine the relationship between LEV concentrations and its therapeutic response, and the effect of LEV on IL1-beta concentrations in patients with epilepsy.

Methods: Patients on monotherapy (n=7) or polytherapy (n=15) with LEV for their seizures management were included. Blood samples of each patient were collected: just before LEV intake, 1 hour, 2 hours, 4 hours and 8 hours following the last dose. Serum LEV concentrations were measured by liquid chromatography mass spectrometry and IL1-beta concentrations by chemiluminescent immunometric assay. Concentration to dose (C/D) ratio values was used for analyses. LEV concentrations were compared between responders (≤1 seizure/month) and non-responders (>1 seizure/month) and patients with or without adverse reactions. IL1-beta concentrations before and at 2 hours following LEV ingestion were compared in order to detect the effect of the increase in serum LEV concentration on IL1-beta.

Results: Although there was no change in LEV (C/D) ratio or LEV maximum concentration (Cmax)/D ratio of the responders and non-responders, the C/D ratio following 1 hour of LEV intake (2.17±0.59 kg.day/L) and Cmax/D ratio (2.25±0.56 kg.day/L) in the patients with adverse effects was significantly higher than for the patients without adverse effects (1.09±0.12 kg.day/L and 1.49±0.14 kg.day/L respectively). A statistically significant decrease was found in the IL1-beta concentration to LEV (C/D) ratio with the increase in LEV concentration in patients on LEV monotherapy.

Conclusion: The possible relationship between LEV Cmax and its therapeutic response or IL1-beta concentrations may be an importance indication of LEV antiepileptic efficacy. Consequently, monitoring LEV Cmax values may enhance LEV adherence because patients would be less likely to develop adverse effects.

Key words: Antiepileptic drugs; seizure frequency; adverse effect; cytokine

1. Introduction

Levetiracetam (LEV) is a broad spectrum antiepileptic drug (AED) which is used for focal epilepsy, myoclonic and tonic-clonic seizures. Patients on LEV therapy can show adverse effects such as dizziness, nasopharyngitis, affective symptoms, aggression, somnolence or anxiety (Bootsma et al., 2007; Hwang et a., 2014; Verrotti et al., 2015). LEV acts primarily by preventing neurotransmitter release through the synaptic cleft by binding to a synaptic vesicular protein, SV2A (Lynch et al., 2004). It has also been reported to inhibit cytokine levels such as IL1-beta, IL2 and IL6 in in-vitro experiments and this anti-inflammatory effect has been considered to be a part of its mechanism of action (Haghikia et al., 2008; Himmerich et al., 2013; 2014).

The relationship between serum drug concentration and therapeutic response has led to the use of therapeutic drug monitoring (TDM) to be an effective tool for guiding dose adjustments of patients with epilepsy on monotherapy or polytherapy with AEDs (Patsalos et al., 2008). For routine implementation of TDM, minimum serum concentration (Cmin or trough) is measured and correlated with the clinical outcome of patients. Therefore, blood samples collected at the end of the dosing interval are used following at least 4 to 5 elimination half-life values of the AEDs (Eadie, 1998). The association of AED serum concentration with the clinical outcome of the patient is based on the target reference range which guides optimal serum drug concentration for maximum clinical effect on seizure control and minimum toxicity (Patsalos et al., 2008). Furthermore TDM is helpful in identifying drug-drug interactions, drug non-compliance, identifying occurrence of adverse effects, the effect of genetic polymorphisms and for managing drug overdose. Genetic polymorphisms can affect the pharmacokinetics or pharmacodynamic characteristics of drugs can alter their therapeutic efficacy. The relationship between polymorphic genes affecting metabolyzing enzymes such as CYP2C9, CYP2C19 or UGT1A4 and serum concentrations of AEDs , the effect of human leukocyte antigen (HLA) alleles and increased risk of idiosyncratic adverse drug reactions or the impact of

pharmacogenetics (ABCB1) on AED resistance have been widely investigated (Balestrini et al., 2018; Gulcebi et al., 2011; Petrenaite et al., 2018). In particular polymorphic metabolism enzymes can directly affect the serum concentration of drugs and can result in therapeutic failure or enhance drug related adverse and toxic effects.

Various LEV reference ranges have been reported including 3 to 34 mg/L, 12 to 46 mg/L or 20 to 40 mg/L and could be attributed to different patient populations being studied (Patsalos, 2003; Patsalos et al., 2008, Stepanova and Beran, 2014). LEV TDM is particularly helpful for the dose adjustments of elderly patients or patients with renal failure related with the increase in the elimination half-life values of LEV (Aldaz et al., 2018). Although LEV is generally not considered to be involved in clinically significant pharmacokinetic drug-drug interactions, consequent to the fact that it is not metabolized *via* CYP450 enzymes and is not plasma protein bound, there are some reports indicating a moderate effect of enzyme-inducing AEDs on serum LEV concentrations (Aldaz et al., 2018; May et al., 2003; Patsalos et al., 2003).

Despite LEV having favorable pharmacokinetics and tolerability characteristics, there are patients with poor therapeutic response with uncontrolled seizures or adverse effects (Bootsma et al., 2007; Lee et al., 2013; Rhee et al., 2017). According to the consensus proposal of ILAE commission on therapeutic strategies for drug resistant epilepsy, one case with LEV was shown as an example for the 'treatment failed' category (Kwan et al., 2010). Particularly behavioral or mood changes have been reported to be associated with intolerability of LEV and a subsequent dose reduction was suggested for the patients with behavioral problems (Chen et al., 2017). The majority of the results investigating the relationship between serum level of LEV and therapeutic response in the adult or pediatric patients with epilepsy showed non-significance (Lancelin et al., 2007; Sheinberg et al., 2015). However these studies included only the trough concentrations of LEV which were measured at the end of the dosing interval. In the present study we aimed to determine: 1) the relationship between LEV concentration and its

therapeutic response for not only serum trough LEV concentration but also for the LEV concentrations measured at subsequent time points: 1 hour, 2 hours, 4 hours and 8 hours following the last dose in patients with epilepsy; 2) the effect of LEV on IL1-beta concentration in patients on monotherapy with LEV by comparing the two IL1-beta concentrations measured just before LEV ingestion and 2 hours following the last dose.

2. Material and Methods

Patients (18-50 years of age) on monotherapy (n=7) or polytherapy (n=15) with LEV for at least one month for the management of their seizures and attending the epilepsy outpatient clinic of the Department of Neurology at Healthsciences University, Medical Faculty, were included. The clinical features of the patients are presented in Table 1 and includes seizure types, electroencephalography (EEG) findings and magnetic resonance (MR) imaging findings. The demographic characteristics of the patients are shown in Table 2. LEV dose, use of other AEDs, seizure frequency and adverse effects were recorded from face to face interview of the patients and also from their hospital notes. Patients who did not want to participate in the study, patients with chronic hepatic or renal disease, with poor LEV compliance or with severe psychiatric disorders were excluded from the study. This study was carried out with the approval of the Marmara University Ethical Committee (MAR-YC-2007-0159). Written informed consent was obtained from all participating patients.

2.1 Measurement of serum LEV and IL1-beta concentrations

Venous blood samples were used for the measurement of serum LEV and IL1-beta concentrations. The blood samples were collected at: just before LEV intake (trough concentrations), 1 hour, 2 hours, 4 hours and 8 hours following the last dose representing steady state LEV concentrations. Sera were prepared by centrifugation of blood samples (4700 G-force for 10 min) at 10 minutes following the collection. Serum samples were transferred to Medical Pharmacology Department of Marmara University *via* cold chain and stored at -80 C

until measurement of LEV and IL1-beta content. LEV concentrations were determined by liquid chromatography mass spectrometry (Shimadzu LC-20 AB Sciex 3200 Qtrap) using a commercial kit (Chromsystems-MassTox Antiepileptic Drugs) exactly as per manufacturer's instructions. The measurement/calibration range was 1.1-82.1 mg/L and the limit of quantification was 1.1 mg/L. IL1-beta concentrations were measured by chemiluminescent immunometric assay (SIEMENS-IMMULITE 1000) exactly as per manufacturer's instructions. The calibration curve with 0-1000 pg/ml.

2.2 Assessment of the relationship between serum LEV concentrations and therapeutic response

Seizure frequency of the patients per month and adverse effects of LEV were evaluated for the assessment of therapeutic response to LEV. Patients on LEV therapy were divided into groups. Patients who were seizure free or had 1 seizure per one month were classified as 'responders' whereas patients with more than 1 seizure per month were considered to be 'non-responders'. The recorded adverse effects of LEV were used for evaluation of the relationship between serum LEV concentrations and development of adverse effects. LEV concentration (mg/L) to dose (mg/kg/day) (C/D) ratio values and Cmax of LEV were compared between responders (≤1 seizure/month, n=11) and non-responders (>1 seizure/month, n=11) and between patients with (n=6) or without (n=16) adverse effects. The C/D ratio was calculated by dividing each measured serum concentration of LEV (mg/L) by the total daily dose (mg/kg).

2.3 Assessment of the effect of LEV on IL1-beta concentrations

The effect of LEV on IL1-beta concentration was determined in the monotherapy patients (n=7) in order to eliminate the possible influences of concurrent AEDs. Only blood samples collected just before LEV ingestion and at 2 hours following last dose were used for this analysis. IL1-beta concentrations matched with Cmin and Cmax level of LEV were used in order to indicate the potential decreasing effect of LEV on IL1-beta concentration. The serum

concentration of IL1-beta (pg/mL) to LEV (C/D) (kg/L.day) ratio values were used for the analysis of the effect of LEV on IL1-beta concentration.

2.4 Statistical analysis

The results were expressed as ‘‘mean±SEM’’ and statistically evaluated by analysis of variance (ANOVA) (GraphPad Software Prism 4.0, San Diego, USA). Two-way ANOVA with repeated measures followed by the post-hoc Bonferroni test was used to analyze the statistical significance of C/D ratio values of LEV between responders and non-responders and between patients with or without adverse effects. Student’s *t*-test was used for evaluating the significance of the difference of the mean Cmax LEV levels between responders and non-responders and between patients with or without adverse reactions. The comparison of IL1-beta/(C/D) ratio values in the monotherapy patients was analyzed with Student’s *t*-test. The level of statistical significance was considered to be $p < 0.05$.

3. Results

Patients on monotherapy (n=7) or polytherapy (n=15) with LEV had different types of epilepsies such as idiopathic generalized epilepsy, myoclonic epilepsy or mesial temporal lobe epilepsy. The mean daily LEV dose of the 22 patients was 23.5 ± 2.2 mg/kg/day and the mean C/D ratio of LEV at the measured 5 time points were as follows: just before LEV intake (trough) = 13.67 ± 1.5 kg.day/L and 1 hour: 28.27 ± 2.8 kg.day/L; 2 hours: 29.54 ± 2.8 kg.day/L; 4 hours: 26.53 ± 2.3 kg.day/L and 8 hours: 17.91±1.7 kg.day/L following the last dose of LEV. Mean Tmax of LEV which shows the duration to reach LEV Cmax (29.5 ± 2.8 mg/L) was 2 hours. Of the 22 patients, 11 were defined as ‘responder’ while the other 11 patients were defined as ‘non-responder’ according to their monthly seizure frequency. Six patients on monotherapy or polytherapy with LEV had adverse effects such as dizziness, drowsiness or behavioral disturbances whilst16 patients were without adverse effects. Patient demographics are shown in Table 1.

Although LEV C/D ratios during monotherapy were generally higher than ratios in polytherapy patients, they were not statistically different. Therefore the two patient groups were combined for the evaluation of the relationship between LEV concentration and its therapeutic response. Although there was no change in LEV C/D ratio or LEV maximum concentration of the responders and non-responders (Fig. 1), comparison of LEV C/D ratio following 1 hour of LEV ingestion in the patients with or without adverse effects (2.17 ± 0.59 kg.day/L and 1.09 ± 0.12 kg.day/L, respectively) was found to be statistically significant ($p < 0.05$, Two-way ANOVA, post-hoc Bonferroni test, Fig. 2A). Cmax/D ratio value for LEV in the patients with adverse effects (2.25 ± 0.56 kg.day/L) was significantly higher than the Cmax/D ratio value for LEV in the patients without adverse effects (1.49 ± 0.14 kg.day/L) ($p < 0.05$, Student's t test, Fig. 2B). The trough LEV concentration before LEV intake and maximum serum LEV concentration were detected in the monotherapy patients *via* subsequent measurements of serum LEV concentration at 5 time points (Fig. 3A). IL1-beta/(C/D) ratios were calculated at the end of the dose interval and at 2 hours following LEV intake (Fig. 3B) when serum LEV concentrations were maximum (Fig. 3A). A statistically significant decrease in the IL1-beta/(C/D) ratio with an increase in LEV concentration was observed in the monotherapy patients ($p < 0.05$, Student's t test, Fig. 3). Serum IL1-beta concentrations and LEV C/D ratio values before LEV intake and at 2 hours following LEV intake of the monotherapy patients are presented in Table 3.

4. Discussion

In the present study, LEV concentrations measured at 5 time points were used to investigate the effect of LEV on seizure frequency and adverse effects. To our knowledge this is the first time this approach has been used and we report that LEV adverse effects were

associated with a significant increase in dose-corrected LEV serum concentration. Furthermore, we observed a decrease in IL1-beta concentration with increasing LEV concentrations.

The first critical finding of the present study is the detection of a possible relationship between the increase in the LEV concentration in the first hour and Cmax of LEV and occurrence of adverse effects. Although there was no difference in LEV concentrations at these 5 time points for its effect on seizure frequency, the mean LEV C/D ratio at 1 hour following LEV ingestion in the patients with adverse effects was shown to be significantly higher than the patients without adverse effects. There are clinical studies indicating lack of a relationship between serum LEV concentrations and its effect on seizure frequency or its adverse effects (Lancelin et al., 2007; Rhee et al., 2017). There was a wide variability in the relationship between trough LEV concentration and clinical outcomes of the adult patients with epilepsy (Lancelin et al., 2007). Similar to adult patients, there was no correlation between trough LEV concentration and its therapeutic response including seizure control and adverse effects, in the pediatric patients with epilepsy (Rhee et al., 2017). The results were also reported not to be affected by other characteristics such as type of the epilepsy seizure, other AEDs, gender or age of the patients. However, these studies are based on serum trough concentrations which correlated Cmin of LEV to its therapeutic response. We also compared the Cmax LEV concentrations of the patients with or without adverse effects. Patients with adverse effects had significantly higher Cmax/D ratio of LEV than the patients without. These results show that the increase in the LEV concentrations 1 hour following the last dose and Cmax of LEV can be valuable indicators for onset of the adverse effects. Measurement of "individualized reference concentration", which would indicate optimal concentration for the best response to an AED in each patient, was highlighted to be possibly useful to prevent adverse reactions or drug-drug interactions (Perucca, 2005). Therefore, follow up of the patients individually, with Cmax of

LEV rather than its trough concentration may be applied as a part of TDM in order to avoid its unwanted effects and thereby improve the quality life of the patients.

The second major finding of this study relates to the potential anti-inflammatory effect of LEV as measured by the marker IL1-beta. The mean IL1-beta/(C/D) ratio of the patients before LEV intake was significantly lower than the values 2 hours following the last dose. There are important findings showing the role of inflammatory processes accompanying to epileptic activity (Vezzani and Granata, 2005; Vezzani et al., 2011). Among proinflammatory mediators, up regulation of IL1-beta, in astrocytes and microglia, was found to play a key role for induction of seizures in experimental and clinical studies and also was considered to be a potential biomarker for epileptogenesis and represent a new target strategy for epilepsy therapy (Balosso et al., 2008; Ravizza et al., 2006, 2008). There is limited literature investigating the anti-inflammatory effect of LEV in the patients with epilepsy (Guenther et al., 2014). Chronic LEV administration was found to have no effect on IL1-beta, IL6 or TNF-alpha concentrations in patients (n= 21) with active epilepsy (Guenther et al., 2014). In contrast, the present study detected an effect of LEV concentration on IL1-beta concentration. The difference may be attributable to the fact that Guenther et al., (2014) measured trough LEV concentrations during chronic administration whilst in the present study peak (Cmax) LEV concentrations were measured. The comparison of IL1-beta levels between trough LEV and maximum LEV by using IL1-beta C/LEV(C/D) ratio values pointed out the decreasing effect of LEV with the increase in its serum concentration. Therefore LEV may show its influence on IL1-beta level only for a short duration when it reaches to its maximum serum concentration. Other cytokines were not included and only the results of monotherapy patients were used in order to discard the potential effects of the other AEDs.

Interestingly although mean LEV C/D ratio values were lower in the polytherapy patients than in the monotherapy patients, there was no statistical difference. The reason for

this observation is probably because the monotherapy (n=7) and polytherapy (n=22) groups comprised of small numbers of patients. Typically, patients on polytherapy regimens and co-prescribed enzyme inducing AEDs (such as our patients) a decrease serum LEV concentrations of 20-30 % can be expected (Patsalos, 2016).

In conclusion monitoring of LEV Cmax concentrations may be a good indicator as to whether or not patients will present with adverse effects. Large population based future studies are needed to corroborate these data and to further understand the underlying mechanisms of the individual differences in therapeutic response to LEV.

Conflict of Interest

None of the authors has any conflict of interest to disclose.

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Figure legends

Figure 1. Comparison of serum LEV concentration between responder and non-responder patients: A. LEV C/D ratio values (Two-way ANOVA, post-hoc Bonferroni test); B. LEV Cmax/D ratio values (Student's t test).

Dashed line arrow shows time of 'LEV intake'. Blood samples were collected just before LEV intake (0) and at1 hour, 2 hours, 4 hours and 8 hours following LEV intake.

Figure 2. Comparison of serum LEV concentration between patients with or without adverse effects: A. LEV C/D ratio values ($p < 0.05$, Two-way ANOVA, post-hoc Bonferroni test); B. LEV Cmax/D ratio values ($p < 0.05$, Student's t test).

Dashed line arrow shows time of 'LEV intake'. Blood samples were collected just before LEV intake (0) and at 1 hour, 2 hours, 4 hours and 8 hours following LEV intake.

Figure 3. The effect of serum LEV concentration on IL1-beta concentration: A. Serum LEV concentrations of the patients on monotherapy with LEV at 5 different time points. (Two-way ANOVA, post-hoc Bonferroni test); B. Comparison of IL1-beta/D ratio values just before LEV intake (minimum LEV concentration) and at 2 hours following LEV intake (maximum LEV concentration) in the monotherapy patients ($p < 0.05$, Student's t test).

Dashed line arrow shows time of'LEV intake'. Blood samples were collected just before LEV intake (0) and at 1 hour, 2 hours, 4 hours and 8 hours following LEV intake. IL1-beta concentrations were measured just before LEV intake and 2 hours following LEV intake.

Table 2. Demographic characteristics of the patients.

Patient Number	Age (year)	Gender Woman/Man	LEV Dose (mg/kg/day)	Adverse Effects	Seizure Frequency	Polytherapy
1	36	W	63	-	2-3/year	Zonisamide
2	62	M	70	-	1/week	Carbamazepine Zonisamide
3	24	M	69	-	2/month	-
4	22	W	52	-	3-4/month	Clonazepam
5	43	W	63	-	3-4/month	Carbamazepine
6	21	W	93	Irritability, nervousness	1-2/year	Valproic acid
7	36	W	56	-	3-4/week	Oxcarbazepine Lamotrigine
8	36	M	65	-	3-4/week	Valproic acid
9	21	W	45	Drowsiness, dizziness, nervousness	2-3/month	Valproic acid Carbamazepine
10	30	W	75	Dizziness, nervousness	2-3/year	-
11	19	M	100	Nervousness	2/month	Oxcarbazepine Zonisamide
12	37	M	90	-	5-6/year	-
13	20	W	45	-	1-2/year	Valproic acid
14	52	W	85	-	1-2/week	Carbamazepine Zonisamide
15	19	W	62	-	1/3-4 years	-
16	29	M	82	-	1/month	-
17	29	M	70	-	1-2/week	Oxcarbazepine Valproic acid
18	45	W	71	-	1/year	-
19	22	M	130	Irritability, nervousness	3/year	Valproic acid
20	28	M	82	-	1/2 years	Carbamazepine Lamotrigine
21	28	W	62	Irritability, nervousness	1/3 years	-
22	28	M	92	-	2/month	Carbamazepine Zonisamide

Table 1. Clinical features of the patients.

Patient Number	Seizure Type	EEG Signals	MR Imaging Findings
1	Focal onset, focal to bilateral tonic-clonic	Left temporal spike-wave (interictal)	Left mesial temporal sclerosis
2	Focal onset, focal to bilateral tonic-clonic	Left temporal spike-wave (interictal)	Normal
3	Focal onset, cognitive	Bilateral fronto-central sharp, slow wave (interictal)	Normal
4	Focal onset, focal to bilateral tonic-clonic	Left lateralized epileptiform discharges	Normal
5	Focal onset, automatisms	Right temporal lobe focal seizure (ictal)	Right mesial temporal sclerosis
6	Generalized onset, tonic-clonic	3-4 Hz generalized spike-wave	Normal
7	Focal onset, focal to bilateral tonic-clonic	Left temporal spike-wave (interictal)	Left mesial temporal sclerosis
8	Focal onset - tonic	Right frontal sharp activity (interictal)	Normal
9	Focal onset, automatisms	Left temporal lobe - focal seizure (ictal)	Left mesial temporal sclerosis
10	Focal onset, behavior arrest	Normal	Normal
11	Focal onset - tonic	Left temporal slow wave (post-ictal)	Right temporal encephalocele
12	Generalized onset, tonic-clonic	Generalized spike-wave discharges (interictal)	Normal
13	Generalized onset, tonic-clonic	3-4 Hz generalized spike-wave	Normal
14	Focal onset - tonic	Right frontal-temporal operculum - epileptic seizure (ictal)	Periventricular gliosis
15	Generalized onset, non-motor	Generalized theta paroxysms	Normal
16	Focal onset - tonic, left frontal	Left frontal spike-wave discharges (interictal)	Post-traumatic encephalomalacia, left > right frontal
17	Focal onset, automatisms	Right temporal lobe - focal seizure (icttal)	Normal
18	Unknown onset, tonic-clonic	Left parieto-occipital spike, slow wave (interictal)	Normal
19	Generalized onset, myoclonic-tonic-clonic	3-4 Hz generalized spike-wave	Normal
20	Focal onset - tonic	Left temporal lobe - focal seizure (ictal)	Left temporal lobe tumor
21	Generalized onset, tonic-clonic	3-4 Hz generalized spike-wave	Normal
22	Focal onset - tonic	Generalized fast activity, spike-wave discharges (post-ictal)	Right hemisphere encephalomalacia

Table 3. Serum IL1-beta concentrations and LEV C/D ratio values before LEV intake and at 2 hours following LEV intake of the monotherapy patients.

Patient Number	IL1-beta (pg/ml)		LEV C/D (kg.day/L)	
	Sample time (h)		Sample time (h)	
	0	2	0 (trough)	2 (Cmax)
3	0.5	0.4	0.7	1.3
10	1.02	0.7	3.3	4.1
12	1.1	0.2	0.7	1.2
15	0.1	0.2	0.4	0.7
16	1.2	0.6	0.7	1.6
18	0.9	1.2	0.9	3.1
21	3.5	2.8	0.6	1.2