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The Potential of Saliva as a Substitute for Blood to Perampanel Therapeutic Drug Monitoring

페람파넬의 치료적 약물모니터링에서 혈액의 대체제로서 타액의 가능성 연구

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김도용

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Abstract

The Potential of Saliva as a Substitute for Blood to Perampanel Therapeutic Drug Monitoring

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Objective: Therapeutic drug monitoring (TDM) of antiepileptic drugs (AEDs) helps optimize drug management for patients with epilepsy. Salivary testing is noninvasive, easy, and has several other advantages. Owing to technical advances, salivary TDM has become feasible for several drugs, including AEDs, and its value has been investigated. Until recently, saliva TDM of perampanel (PER) had not been reported. The purpose of our study was to confirm whether saliva is a biological substitute for plasma in PER TDM.

Methods: Adult patients diagnosed with epilepsy who received PER from August 2018 to March 2019 at Seoul National University Hospital were

enrolled. Total and free PER were measured in simultaneously obtained plasma and saliva samples using LC–MS/MS and HPLC. We examined the correlations between saliva and plasma PER concentrations and whether the use of concomitant medications classified as CYP3A4 inducers affected the correlations.

Results: Thirty patients were enrolled, aged 16 to 60; 10 (33%) were women. Patients received 2 to 12 mg (mean, 6 mg) of PER. The average total and free concentrations of PER were 343.02 (46.6–818.0) and 1.53 (0.51–2.92) ng/ml in plasma and 9.74 (2.21–33.0) and 2.83 (1.01–6.8) ng/ml in saliva, respectively. A linear relationship was observed between the total PER concentrations in saliva and the total and free PER concentrations in plasma (both $p<0.001$; $r= 0.678$ and $r= 0.619$, respectively). The change in the PER concentration caused by the CYP3A4 inducer did not affect the correlation between saliva and plasma concentrations (all $p<0.001$).

Conclusion: The PER concentration in saliva was correlated with that in plasma. This correlation was not affected by CYP3A4 inducers. Our results demonstrate for the first time that PER is measurable in saliva and suggest the potential for the clinical application of the saliva PER TDM matrix.

Keywords: therapeutic drug monitoring; perampanel; saliva; plasma; correlation

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List of Anti–epileptic Drugs' Abbreviations

CBZ: carbamazepine	CLB: clobazam
ESM: ethosuximide	GP: gabapentin
LCS: lacosamide	LEV: levetiracetam
LMT: Lamotrigine	OXC: oxcarbazepine
PB: phenobarbital	PHT: phenytoin
PRG: pregabalin	RFN: rufinamide
TPM: topiramate	VPA: valproate
ZNS: zonisamide	

I. Introduction

1. Perampanel

Perampanel (PER) is an antiepileptic drug (AED) targeting the α–amino–3–hydroxy–5–methylisoxazole–4–propionic acid (AMPA) glutamate receptor on postsynaptic neurons.[1] Glutamate is the primary excitatory neurotransmitter in the central nervous system and is implicated in a number of neurological disorders caused by neuronal over–excitation. The activation of AMPA receptors by glutamate postulated to be responsible for most fast–excitatory synaptic transmission in the brain.[1] PER does not compete with AMPA for binding to the AMPA receptor in vitro, but bound PER is displaced by noncompetitive AMPA receptor antagonists, indicating that PER is a noncompetitive AMPA receptor antagonist. PER inhibits the AMPA–induced (but not NMDA–induced) increase in intracellular calcium concentrations in vitro. PER also significantly prolongs seizure latency in an AMPA–induced seizure model in vivo.[1–5]

PER was approved by the U.S. Federal Drug Administration (FDA) in October 2012.[1] Since 2015, it has been licensed in Korea as an adjunct treatment for partial onset seizures with or without secondary generalized seizures in patients with epilepsy older than 12 years. Its half–life is approximately 105 hours, and thus it is taken once a day. The daily dose is 2–12 mg, and its steady state concentration is reached approximately 2–3 weeks after administration.[2,4,5]

The therapeutic efficacy of PER has been confirmed in current clinical trials: 1) phase 3 clinical trials have been conducted in patients with refractory epilepsy who have experienced uncontrolled partial seizures, despite the administration of 2 or more AEDs in the past 2 years.[6–8] The rate of change in seizure frequency in patients with combined generalized seizures and secondary generalized seizures confirmed that PER was significantly more effective than the placebo. In particular, a dramatic response leading to a seizure–free state has been observed in approximately 5–10% of patients. Adverse reactions that cause the discontinuation of PER include dizziness, somnolence, drowsiness, ataxia, aggression, stuttering, and blurred vision.[1,3] 2) PER shows a linear dose–concentration relationship with the serum PER concentration regardless of the patient's age and gender.[4] 3) Since the majority of PER is metabolized by cytochrome P450 (CYP3A4) in the liver, it has a strong interaction with AEDs targeted by the same enzyme.[10,11] CBZ, OXC, and PHT increase PER clearance, reducing the area under the plasma PER concentration–time curve (AUC) by 67%, 50%, and 50%, respectively.[3]

2. Therapeutic drug monitoring and applications of perampanel

Therapeutic drug monitoring (TDM) is an analysis that measures the concentration of a specific drug to maintain a relatively constant drug concentration in the bloodstream.[9]

For some drugs, the steady state is not maintained simply by administering a standard drug dose. Therapeutic drugs are absorbed, metabolized, utilized, and eliminated at different rates, depending on the patient's age, health status, genetic structure, and interactions with other drugs taken, even when administered at the same amount. The blood concentration of the drug does not exert an effect below a certain concentration (minimum effective concentration, C_{min}), and side effects may occur if it exceeds the maximum effective concentration (C_{max}).[12–14] Therefore, TDM is particularly required for drugs with a narrow therapeutic index, such as AEDs.[13]

Specific indications for the TDM of AEDs listed below.[15]

1. <u>After initiation of AED treatment or after dose adjustment</u>

TDM enables a preselected reference range for the individual patient to be pursued. Blood concentrations should always be measured before a change in the drug dose and after the achievement of the steady state at the new dose.

2. <u>Upon the achievement of the optimum desired clinical response.</u>

Seizure freedom is the optimal outcome, but for many patients, optimum seizure control with minimal adverse effects is more readily achieved. Tin this case, TDM allows the "individual therapeutic range" to be established. Two separate measurements should be performed at a 2–4 month interval to estimate the extent of any variability and to establish the therapeutic concentration for each patient.

3. <u>To determine the magnitude of a dose change.</u>

TDM is particularly important for AEDs that show dose dependent pharmacokinetics (e.g., PHT, CBZ, VPA, GP, and RFN). Blood concentrations should always be measured before a change in the drug dose and after the achievement of the steady state at the new dose.

4. When toxicity is difficult to differentially diagnose or when toxicity is difficult to assess in the clinic.

Concentration–related AED toxicity is more readily identified and is particul arly helpful when young children or patients with a mental disability are being evaluated. Elevated plasma concentrations of many AEDs can exacer–bate seizures, and TDM will assist the differential diagnosis in the case of a patient with epilepsy who is admitted with seizures.

5. When seizures persist despite the prescription of an adequate/typical dosage.

In this case, TDM may identify a fast metabolizer or a patient who is non compliant with his/her AED medication.

6. When pharmacokinetic variability is expected.

Pharmacokinetic variability encompasses a broad category of patients, including children and the elderly, pregnant woman, patients with hepatic diseases, renal diseases, or various pathologies, patients who have undergone surgery, and drug–drug pharmacokinetic interactions.

7. When a change in the formulation occurs.

A change in formulation includes immediate–release to sustained–release formulations and brand–name to generic and generic to generic switches. Blood concentrations must be measured before a change in the formulation. and after the achievement of the steady state with the new formulation.

8. The clinical response has unexpectedly changed.

The cause of the change will be readily identifiable among many factors.

9. Poor compliance is suspected.

Recent noncompliance is readily identifiable. However, long–term compliance or variable compliance cannot be identified.

The measurement of free drug concentrations is important, particularly for AEDs with high–affinity protein binding, such as PER, because only free drugs that are not bound to proteins cross the blood–brain barrier and exert a pharmacological effect. Measurement of the free levels of AEDs that highly bind to proteins is particularly important in patients with the following conditions: 1) alteration of drug–protein binding interactions due to uremia, chronic liver disease, or hypoalbuminemia (burns, advanced age, pregnancy, AIDS, etc.), and 2) drug–drug interactions where a strongly bound drug decreases the binding of an AED to a protein by displacement.[16–18]

Patsalos et al.[19] reported a reference range of 180–980 ng/ml for plasma PER concentrations, and Gidal et al.[4] predicted a therapeutic range of greater than approximately 70 ng/ml for steady–state plasma concentrations. The toxic level of PER associated with adverse events has not yet been established; however, significantly increased PER concentrations have been observed in patients experiencing specific adverse events (gait disturbance, irritability, somnolence, etc.) compared to patients without these adverse events.[4]

3. Biological matrices for TDM

TDM is generally performed using blood samples. However, venipuncture has multiple disadvantages: it is an invasive method that causes pain, requires a skilled phlebotomist or nurse, and must be performed in a medical institution. Therefore, TDM has been performed using various biological matrices.[15,19]

1) Dried blood spot (DBS)

A DBS, which has a simple sampling method and only requires a small amount of blood, has been used to monitor the levels of PHT, PB, LEV, LCS, TPM, ESM, LMT, RFN, ZNS, and OXC and its active metabolite 10–OH–monohydroxy–carbazepine.[20–22] The ability to perform sampling in children or patients with reduced consciousness is an advantage, but it also has disadvantages such as its invasive nature and the inability to obtain results from some blood spots.[19]

2) Tears

A study of PB, CBZ, PHT, ESM, VPA, and other drugs reported the free, non–protein–bound drug concentrations detected in tears.[23–28] In particular, Nakajima et al.[28] reported a strong correlation between tears and plasma concentrations with only a small amount (3–4 μl) of sample analyzed using gas chromatography/mass spectrometry. The collection of tear samples requires the use of a capillary tube, which is somewhat cumbersome, and has disadvantages such as patient rejection when using methods such as cigarette smoke and/or sniffing formaldehyde to induce brisk tearing.[15]

3) Cerebrospinal fluid (CSF)

The CSF concentrations of many AEDs reflect the free, non–protein–bound drug concentrations. On the other hand, a nonlinear curve is observed for drugs

that are distributed throughout the body, such as PRG and GP.[29–33] The greatest disadvantage of CSF is the invasive collection methods, such as lumbar puncture.

4) Sweat

Although sweat has been less frequently studied than other fluids, several studies of drug concentrations in sweat have been reported for the first generation of AEDs.[34,35] According to Parnas et al.,[34] PHT, CBZ, and PB are all present in sweat, and the concentration of PHT corresponds to the free, non–protein–bound fraction in serum and is independent of sweat flow. Sweat has been rarely studied in recent years due to disadvantages such as large fluctuations in concentrations, depending on the collection method.[15]

5) Hair

Hair has the characteristic of continuous growth. The hair root will be exposed to the steady–state drug concentration through the blood, resulting in a constant concentration of the drug along the hair shaft. AEDs reported to be transported in hair include CBZ, PHT, VPA, OXC, and LMT.[36–40] The disadvantage of hair analyses is that the amount of accumulated drug changes due to many factors, such as dyeing, melanin content, and drying, and thus substantial fluctuations in the concentration have been observed.[15]

6) Saliva

The use of saliva for PER TDM would be a great advantage due to its simple collection method, which is cost effective, painless, and noninvasive. Saliva mainly consists of water (>99%) but also includes small amounts of organic and inorganic components. Certain drugs are considered organic components, which also include mucins, prolines, enzymes (amylase, lipase, peroxidase etc.), and histatins. While inorganic components such as electrolytes are actively transported to saliva, organic components (including drugs) enter saliva through passive diffusion, reflecting the concentration in blood.[41] Accordingly, studies have been actively conducted on saliva as an alternative biological fluid for TDM of AEDs since the 1970s **(Table 1).** However, the disadvantages of saliva collection include oral conditions, diurnal variations, and the difficulty of collecting samples from unconscious patients.

Table 1. Study of the correlation between AED concentrations in saliva and blood

AEDs	Year approved by the US FDA	Correlation coefficient (r^2) between total and free concentrations in the blood and saliva.		Utility of monitoring saliva
		Blood total & saliva	Blood free & saliva	
Brivaracetam (Briviact)[42]	2016	0.97	–	O
Carbamazepine (Tegretol)[43–48]	1968	0.84 – 0.99 0.76 – 0.88†	0.91 – 0.99 0.75 – 0.98†	O
Clobazam (Frisium)[49,50]	1974	0.90 0.93§	– –	O
Clonazepam (Rivotril)[51]	1974	Not Validated		X
Eslicarbazepine acetate (Zebinix)[52–56]	2013	0.99	–	O
Felbamate (Felbatol)	1993	Not Validated		X
Gabapetin (Neurontin)[57]	1993	> 0.7	–	O
Lacosamide (Vimpet)[58,59]	2008	0.84	0.83	O
Lamotrigine (Lamictal)[60–62]	1994	0.81 – 0.94	0.93 – 0.95	O
Levetiracetam (Keppra)[63,64]	1999	0.86 – 0.91	–	O
Oxcabazepine (Trileptal)[65–68]	1999	0.91 – 0.98¶	–	O
Perampanel (Fycompa)	2012	Not Established		–
Phenobarbital (Luminal)[69–75]	1912	0.65 – 0.98	0.64 – 0.99	O
Phenytoin (Epanutin)[76,77]	1998	0.85 – 0.99	0.96 – 0.99	O
Pregabalin (Lyrica)	2004	Not Established		–
Primidone (Mysoline)	1952	0.71 – 0.97	–	O
Rufinamide (Inovelon)[78]	2008	Not Established		O
Stiripentol (Diacomit)	2018	Not Established		–
Tiagabine (Gabitril)	1998	Not Established		–
Topiramate (Topamax)[79]	2006	0.97	–	O
Valproic acid (Epilim)[80–83]	1983	Not Validated		△
Vigabatrin (Sabril)	2009	Not Established		–
Zonisamide (Zonegran)	2000	Not Established		–

†Refers to values for the active metabolite carbamazepine–epoxide.

§Refers to values for the active metabolite *N–desmethyl–clobazam.*

¶Refers to values for the active metabolite *10–hydroxycarbazepine* and a significant correlation was only observed in unstimulated saliva.

4. Considerations for salivary AED measurements

Saliva collection methods are divided into two types: 1) stimulating methods that induce the production of saliva with citric acid or by chewing inert parafilm balls and 2) nonstimulating methods for collecting naturally produced saliva.[84,85] In the latter case, the process of rinsing the mouth prior to collection is essential to avoid errors in the results that may be caused by residues in the mouth. In addition, saliva collection may not be appropriate in patients with conditions such as cavities and bleeding gums, because outlier values for drug concentrations may occur, depending on the condition of the oral cavity.[15,86]

Patsalos et al.[19] emphasized that saliva sample information such as sampling time and a meticulous dosage history is important for saliva AED TDM to provide useful knowledge. In addition, sampling is recommended in 5 half–lives to reach a steady state after starting treatment or changing the dose. For AEDs with long half–lives such as PER and ESM, fluctuations in drug concentrations in both serum and saliva during the dosing interval are able to be neglected, and thus sampling can be performed at any time. On the other hand, for large numbers of AEDs with short half–lives, such as LCS, OXC, and PRG, a standardized sampling time must be established. The ideal saliva sampling time for all AEDs is in the morning immediately before the next oral dose is consumed (trough).[15]

Similar to blood, salivary AED analyses have been performed using chromatography methods and immunoassays.[87–91] Unlike blood, saliva should have a very low calibration range, particularly for AEDs with a high level of

protein binding (since the saliva concentration reflects the free level of most AEDs). Considering the high viscosity of saliva, additional procedures such as ultra–centrifugation may be necessary.

5. **Objectives**

Because of the simplicity of saliva collection, the author hypothesized that if saliva was collected from patients who took PER immediately after the presentation of side effects or seizures, the drug level at the time of the event would be able to be assessed.

The purpose of this study, which is the first step in testing this hypo–thesis, was to determine whether saliva is a potential substitute for plasma in the TDM of PER by evaluating 1) the correlation between saliva and plasma PER concentrations and 2) the effects of CYP 3A4 inducers on the correlation between saliva and plasma concentrations of PER.

II. Methods

1. Study subjects

Patients with epilepsy who were taking PER (FYCOMPA®, Eisai, Japan) and who visited Seoul National University Hospital (SNUH) between August 2018 and March 2019 were included in this study.

Thirty adult patients whose dose was not changed for 2–3 weeks and who agreed to participate in the study were enrolled. We investigated the time point at which the last PER dose was taken, the dose, any concomitant AEDs taken, the food intake before blood sampling and the smoking status during the study enrolment period. The study was approved by the Institutional Review Board of SNUH (IRB no. 1805–113–948), and written informed consent was obtained from all patients.

2. *Sample collection and storage*

Blood and saliva samples were simultaneously collected from patients. At least 5 ml of blood were collected in sodium–heparin tubes (BD Bioscience, USA), and approximately 1–3 ml of saliva were collected in untreated polypropylene tubes (BD Bioscience, USA). The method for saliva collection was standardized and was applied equally to all subjects. Briefly, the mouth was rinsed with water before saliva collection, and the unstimulated saliva was collected using the spitting method.[84,85] Within 3 hours of collection, samples were stored at -70 ℃ (saliva was stored without

any manipulation; blood was centrifuged at 1320 g for 5 minutes at 4 ℃ to separate plasma, which was subsequently stored) until analysis. The storage period did not exceed three months.

3. *Analysis of drug concentrations*

Free and total PER concentrations in plasma and saliva were measured independently using high–performance liquid chromatography (HPLC) (1200 series, Agilent Technologies, Santa Clara, CA, USA) coupled with tandem mass spectrometry (MS/MS) (API3200, Applied Biosystems, Waltham, MA, USA).

To determine the total concentration of PER, 50 µl of plasma or saliva was mixed with 50 µl of an internal standard (100 ng/ml trazodone in 50% methanol), and 300 µl or 200 µl of acetonitrile was then added. The mixture was centrifuged at 9425 g; for 5 minutes at 4 ℃, and 2 µl of the supernatant was injected into the LC–MS/MS system. 1 ml of plasma or saliva was added to an Amicon® ultra–30k centrifugal filter (Merck Millipore, Burlington, MA, USA), and filters were centrifuged at 1910 g; for 20 minutes at 37 ℃ to determine the free concentration of PER, this method was modified from previously reported protocols.[18] The filtered solutions were analysed using the same method as described for the measurement of the total PER concentration.

Chromatographic separation was achieved on an Agela Venusil ASB C8 column (3 µm, 150 Å, 50 x 2.1 mm) (Agela Technologies), and the

column temperature was maintained at 25°C. The mobile phases consisted of 2 mM ammonium formate containing 0.1% formic acid and 100% acetonitrile containing 0.1% formic acid, and the separations were conducted at a flow rate of 0.2 ml/min. The MS/MS unit was operated in positive electrospray ionization mode. For the optimization process, we tested 3 transitions and selected the most appropriate transition for analysis according to our SOPs. The table shown below provides an example of the 3 transitions. Therefore, PER and the internal standard were detected in multiple reaction monitoring mode at m/z values of 349.96 → 219.10 and 372.10 → 176.20, respectively.

PER (m/z)	IS (m/z)
349.96 → 219.1	372.1 → 176.2
349.96 → 247	372.1 → 147.9
349.96 → 77.1	372.1 → 78.2

According to FDA guidelines,[92] selectivity analysis that separates and quantifies target analytes when other materials coexist in the biological sample, should be performed using six different biological samples from plasma and saliva. The lower limit of quantification (LLoQ) should be set to a value at which the interference peak area from the six biological samples is 20% or less of the peak area of the lowest quantitated concentration of the analyte (5% or less for the internal standard material). According to this guideline, we conducted selectivity analysis of plasma and saliva samples, and the results were 0.5 and 1.0, respectively. In addition, we also considered suspected concentrations of PER in plasma and saliva. Based on these

considerations, the LLoQs of PER in plasma and saliva were set to 0.5 ng/ml and 1.0 ng/ml, respectively. In the present study, the PER concentrations in all plasma and saliva samples exceeded the LLoQ values. Moreover, the calibration range of PER was 0.5 ng/ml to 500 ng/ml for plasma and 1.0 ng/ml to 500 ng/ml for saliva. The intraday (interday) precision (coefficient of variation, %CV) was less than 9.054% (4.039%) in plasma and less than 7.03% (4.993%) in saliva. The intraday (interday) accuracy was greater than 103.64% (103.56%) in plasma and greater than 106.4% (105.05%) in saliva.

4. *Data analysis and statistics*

The correlation between saliva and plasma concentrations was determined by calculating the Pearson correlation coefficient. First, we observed whether the total and free concentrations of PER were significantly correlated in plasma and saliva samples. Second, we measured the correlations of the total PER concentrations in plasma and saliva with the free concentrations of PER in plasma and saliva. Finally, the correlations between the total PER concentration in plasma and free PER concentration in saliva and between the total PER concentration in saliva and free PER concentration in plasma were examined.

Because saliva and plasma contain different proteins and because the CYP3A4 enzyme is expressed in salivary glands,[93–95] we investigated whether inducers performed differently in the systemic circulation and saliva. 1) We used Student's t–test to assess differences in plasma and saliva

concentrations in patients treated with or without the CYP3A4 inducer to determine the effect of the inducer on PER concentrations. Patients were divided into the inducer group (those who took a concomitant CYP3A4 inducer, such as CBZ, OXC, and PHT[96,97]) and the noninducer group (those who did not take a concomitant CYP3A4 inducer). The analytical concentrations in each of the two groups were corrected by dividing the values by the daily maintenance dose, which was defined as the corrected PER concentration (PERcorr_conc.). 2) In addition, partial correlations were analysed using the CYP3A4 inducer as a control variable to clarify the genuine correlation between saliva and plasma concentrations. The outlier was set to a value greater of less than 1.5 times the upper or lower quartile based on the median value of PERcorr conc.[98]

The geometric mean value was calculated and analyzed using PERcorr_conc. as raw data to evaluate the impact of inducer. The geometric mean ratio (GMR) was then calculated by dividing the geometric mean of the inducer group by the value of the noninducer group, and a GMR ≤ 0.8 was considered a significant difference.[99] SPSS 25.0 (IBM, Japan) was used for the statistical analyses, and a p–value < 0.05 was considered significant.

III. Results

1. Patient characteristics

Thirty subjects were enrolled (**Table 2 and Figure 1**). The age of the subjects ranged from 16–60 years (33.7 ± 12.8 years), and the sample consisted of 20 males and 10 females. The mean maintenance dose was 6 ± 2.6 mg. The time that had elapsed since the last dose was 10–39 hours (14.4 ± 5.2 hours). Three of the enrolled subjects smoked, and 4 had eaten within 1 hour prior to the collection of blood and saliva. In addition, all but one patient was prescribed concomitant AEDs.

2. PER concentrations in the plasma and saliva samples

The mean concentrations of total and free PER were 343.02 ± 207.67 and 1.53 ± 0.84 ng/ml in plasma and 9.74 ± 6.22 and 2.83 ± 1.66 ng/ml in saliva, respectively (**Table 3**). Free PER accounted for 0.45 % of the total concentration in plasma and for 29.1 % in saliva. A significant difference was not observed between the sexes ($p > 0.05$), and changes in concentrations due to smoking and eating habits were not detected due to the small number of subjects. The total plasma concentration of PER in 1 patient (patient number 16) and the total saliva concentrations of PER in 2 patients (patient numbers 20 and 24) were outliers.

Table 2. Demographic characteristics of patients who underwent concurrent blood and saliva sampling during the steady state period for PER.

ID	Sex	Age	Diagnosis[a]	Dose[b]	Plasma conc.[c] (PERcorr_conc.[d])		Saliva conc.[c] (PERcorr_conc.[d])		Plasma free: total ratio	Saliva free: total ratio	Concomitant AED[e]
					Total	Free	Total	Free			
1	M	24	OLE	10	479 (47.9)	2.89 (0.29)	12.9 (1.29)	5.22 (0.52)	0.006	0.4	OXC 900
2	M	29	TLE	12	607 (50.6)	1.78 (0.15)	19.3 (1.61)	6.38 (0.53)	0.0029	0.33	VPA 1000, LEV 2000
3	F	39	TLE	2	214 (107)	0.788 (0.39)	2.71 (1.36)	1.36 (0.68)	0.0037	0.5	LCS 200
4	M	45	FLE	10	310 (31)	1.45 (0.15)	9.57 (0.96)	1.8 (0.18)	0.0047	0.19	CLB 10, CBZ 800, LEV 2000, LCS 100
5	M	25	FLE	6	641 (106.8)	2.66 (0.44)	14 (2.33)	5.03 (0.84)	0.0041	0.36	VPA 1000, ZNS 200, LEV 1000
6	M	35	GE	4	486 (121.5)	2.21 (0.55)	12.2 (3.05)	5.03 (1.26)	0.0045	0.41	LEV 3000, VPA 2000, CLB 5
7	M	16	GE	4	158 (39.5)	0.606 (0.15)	2.85 (0.71)	1.08 (0.27)	0.0038	0.38	VPA 1250
8	M	29	TLE	8	522 (65.3)	2.13 (0.27)	8.83 (1.1)	2.47 (0.31)	0.0041	0.28	LMT 400, LEV 3000
9	F	57	TLE	8	94.5 (11.8)	0.718 (0.09)	4.42 (0.55)	1.96 (0.25)	0.0076	0.44	CBZ 800, VPA 600
10	M	26	TLE	8	111 (13.9)	0.541 (0.07)	4.3 (0.54)	2.3 (0.29)	0.0049	0.53	OXC 1500, TPM 100, LEV 2000
11	M	19	TLE	4	189 (47.3)	0.573 (0.14)	3.34 (0.84)	1.41 (0.35)	0.003	0.42	VPA 1000
12	F	30	GE	6	818 (136.3)	2.92 (0.49)	14.3 (2.38)	6.8 (1.13)	0.0036	0.48	TPM 25, LEV 2000
13	F	35	TLE	8	263 (32.9)	0.89 (0.11)	3.69 (0.46)	1.11 (0.14)	0.0034	0.3	LEV 1500, LCS 400
14	M	27	OLE	6	460 (76.7)	1.72 (0.29)	12.8 (2.13)	4 (0.67)	0.0037	0.31	CLB 10, VPA 1000, OXC 600, LMT 250, LEV 2000
15	F	33	FLE	4	214 (53.5)	1.56 (0.39)	8.5 (2.13)	3.75 (0.94)	0.0073	0.44	LEV 1000, ZNS 200, LCS 200
16	F	49	FLE	2	440[f] (220[f])	0.775 (0.39)	8.66 (4.33)	2.87 (1.44)	0.0018	0.33	LEV 1500

17	F	23	TLE	4	360 (90)	1.52 (0.38)	9.38 (2.35)	2.37 (0.59)	0.0042	0.25	LMT 300, LEV 1000
18	M	27	GE	4	102 (25.5)	0.56 (0.14)	7.45 (1.86)	1.25 (0.31)	0.0055	0.17	CLB 20, CBZ 800, LEV 2000, LCS 400
19	M	52	TLE	6	325 (54.2)	1.85 (0.31)	11.3 (1.88)	2.87 (0.48)	0.0057	0.25	CLB 5, VPA 1500, OXC 1200, LEV 2000
20	M	36	TLE	2	146 (73)	0.527 (0.26)	11.6[f] (5.8[f])	1.97 (0.99)	0.0036	0.17	–
21	M	25	PLE	6	228 (38)	1.48 (0.25)	7.81 (1.3)	2.26 (0.38)	0.0065	0.29	VPA 1000, OXC 1500
22	F	52	TLE	4	63.1 (15.8)	0.51 (0.13)	2.21 (0.55)	1.02 (0.26)	0.081	0.46	CBZ 1000, VPA 1000
23	M	46	TLE	8	46.6 (5.8)	0.792 (0.1)	5.93 (0.74)	1.02 (0.13)	0.017	0.17	OXC 1200
24	F	18	FLE	8	728 (91)	2.66 (0.33)	33[f] (4.13[f])	3.1 (0.39)	0.0037	0.09	OXC 450, LEV 1000
25	F	28	TLE	6	355 (59.2)	2.69 (0.45)	12.1 (2.02)	5.27 (0.88)	0.0076	0.44	LEV 2000, TPM 200, LCS 400
26	M	57	FLE	4	82.5 (20.6)	0.52 (0.13)	3.39 (0.85)	1.03 (0.26)	0.0063	0.3	VPA 1000, ZNS 200, LEV 3000
27	M	28	FTLE	6	450 (75)	1.31 (0.22)	13.9 (2.32)	2.45 (0.41)	0.0029	0.18	LMT 400, LEV 3000
28	M	19	FLE	6	351 (58.5)	2.06 (0.34)	14 (2.33)	2.61 (0.44)	0.0059	0.19	VPA 1000, LEV 3000
29	M	60	TLE	10	398 (39.8)	2.5 (0.25)	14.3 (1.43)	4.03 (0.4)	0.0063	0.28	LEV 2000
30	M	22	GE	4	649 (162.3)	2.75 (0.69)	3.41 (0.85)	1.01 (0.25)	0.0042	0.3	CLB 20, LEV 1500

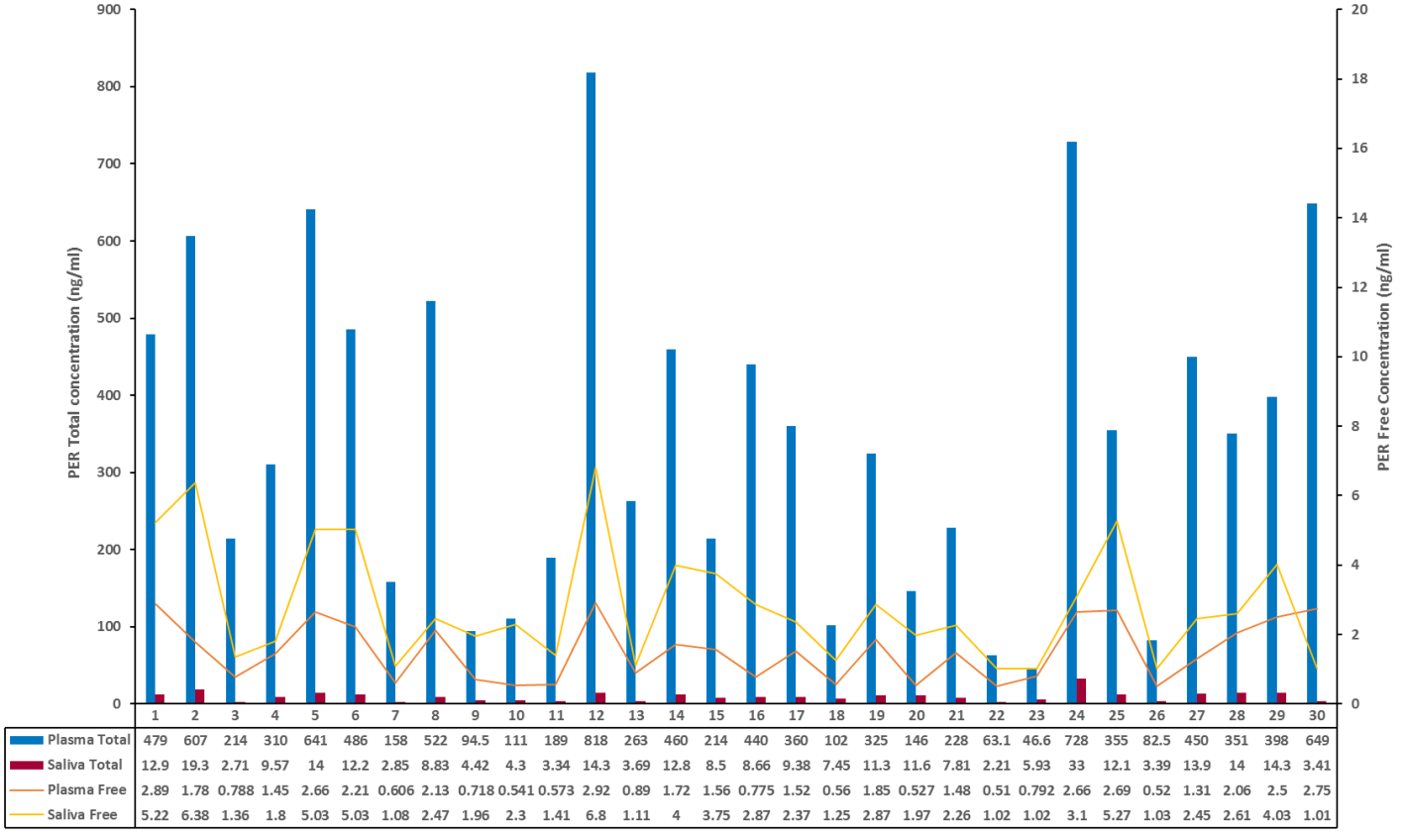
[a]FLE, Frontal Lobe Epilepsy; GE, Generalized Epilepsy; OLE, Occipital Lobe Epilepsy; TLE, Temporal Lobe Epilepsy; PLE, Parietal Lobe Epilepsy
[b]The maintenance dose that was being taken at the time of the drug concentration measurement
[c]ng/ml
[d]Corrected perampanel concentration; concentration(ng/ml), per daily dose(mg/day)
[e]Daily dose(mg/day), AEDs, Antiepileptic Drugs; CBZ, Carbamazepine; CLB, Clobazam; LCS, Lacosamide; LEV, Levetiracetam; LMT, Lamotrigine; OXC, Oxcarbazepine; TPM, Topiramate; VPA, Valproate; ZNS: Zonisamide
[f]Oulier – greater than a 1.5–fold change based on the median PERcorr_conc. of each group (with or without a CYP3A4 inducer) as determined by the interquartile range outlier rule.

Table 3. Plasma and saliva concentrations measured in the enrolled subjects in the clinic.

	Plasma		Saliva	
	Total PER conc.[a]	Free PER conc.[a]	Total PER conc.[a]	Free PER conc.[a]
Mean ± SD	343.02 ± 207.67	1.52 ± 0.85	9.73 ± 9.1	2.8 ± 1.71
Range	46.6 – 181	2.21 – 33	0.38 – 2.92	0.89 – 6.8
Median	338	9.1	1.5	2.41

[a]ng/ml

Figure 1. PER concentrations measured in the subjects



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Plasma Total	479	607	214	310	641	486	158	522	94.5	111	189	818	263	460	214	440	360	102	325	146	228	63.1	46.6	728	355	82.5	450	351	398	649
Saliva Total	12.9	19.3	2.71	9.57	14	12.2	2.85	8.83	4.42	4.3	3.34	14.3	3.69	12.8	8.5	8.66	9.38	7.45	11.3	11.6	7.81	2.21	5.93	33	12.1	3.39	13.9	14	14.3	3.41
Plasma Free	2.89	1.78	0.788	1.45	2.66	2.21	0.606	2.13	0.718	0.541	0.573	2.92	0.89	1.72	1.56	0.775	1.52	0.56	1.85	0.527	1.48	0.51	0.792	2.66	2.69	0.52	1.31	2.06	2.5	2.75
Saliva Free	5.22	6.38	1.36	1.8	5.03	5.03	1.08	2.47	1.96	2.3	1.41	6.8	1.11	4	3.75	2.87	2.37	1.25	2.87	1.97	2.26	1.02	1.02	3.1	5.27	1.03	2.45	2.61	4.03	1.01

3. Correlation between the plasma and saliva concentrations of PER.

Good linear correlations were observed between the total and free concentrations of PER in plasma (p<0.001, r=0.836) and saliva (p<0.001, r=0.607). Additionally, positive correlations between plasma were observed between the total concentrations (p<0.001, r=0.678) and free concentrations in plasma and saliva samples (p<0.001, r=0.710). The correlations between the total concentration in plasma and the free concentration in saliva (p<0.001, r=0.679), and between the total concentration in saliva and the free concentration in plasma (p<0.001, r=0.619) were good. All the significance levels were less than 0.001 (**Figures 2 and 3**). The change in the correlation coefficient attributed to outliers was not significant. (**Table 4**)

Table 4. The correlation coefficients calculated before and after the exclusion of outliers.

	Plasma Total Conc.	Plasma Free Conc.	Saliva Total Conc.	Saliva Free Conc.
Plasma Total Conc.	1			
Plasma Free Conc.	0.836 → 0.866*	1		
Saliva Total Conc.	0.678 → 0.688*	0.619 → 0.684*	1	
Saliva Free Conc.	0.679 → 0.682*	0.710 → 0.710*	0.607 → 0.824*	1
*p < 0.001				

Figure 2. Relationship between the total PER concentrations in saliva with total and free PER concentrations in plasma.

(A) The correlations between the total concentrations in plasma and saliva were measured ($p< 0.001$, r= 0.678). To confirm the association between the plasma and saliva concentrations to assess the influence of the free PER concentration on its direct effect. **(B)** The correlations of total PER concentrations in saliva and free PER concentrations in plasma were determined to examine the direct effect of the free PER concentration, and deemed to be significant ($p< 0.001$, r= 0.619).

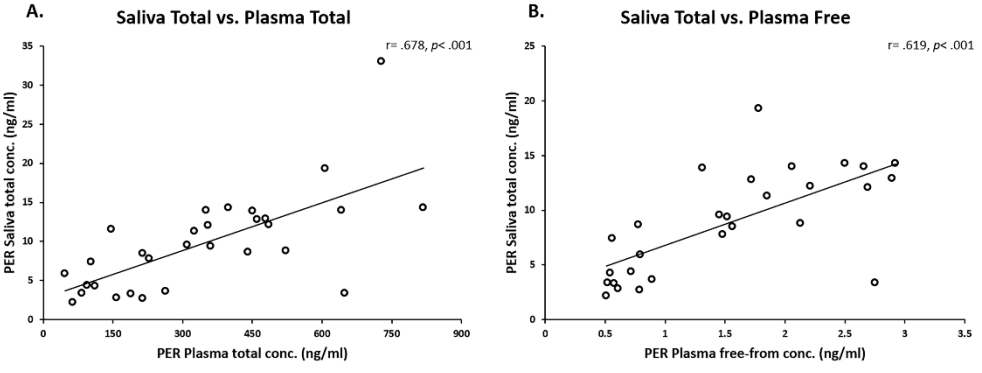
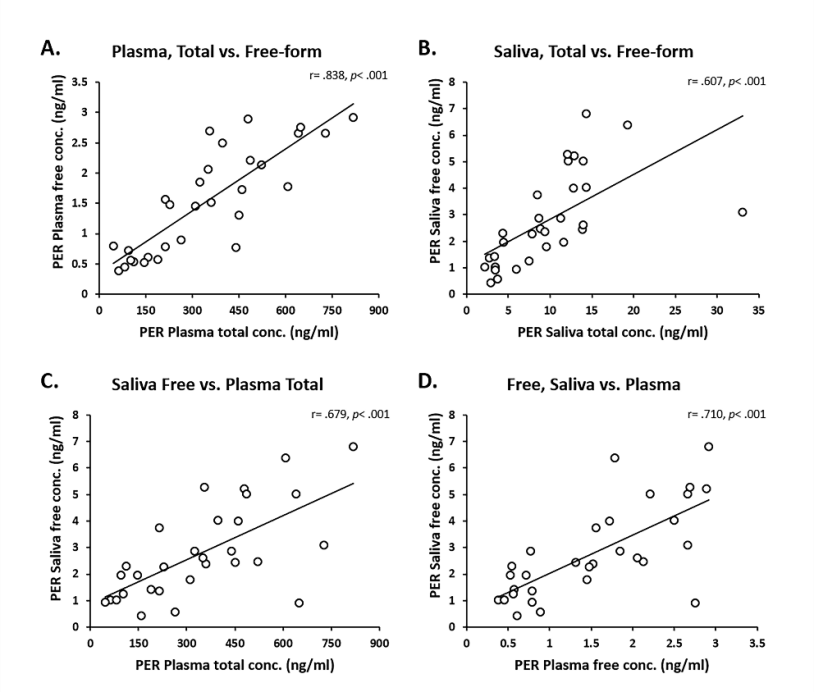


Figure 3. Relationship between PER concentrations in plasma and saliva.

First, we analyzed the correlation between the concentrations of the total and free forms of PER in plasma and saliva. **(A**) The total PER concentration in plasma was significantly associated with the free PER concentration in plasma ($p< 0.001$, r= 0.836), and **(B)** the total and free concentrations of PER in saliva were significantly correlated ($p< 0.001$, r= 0.607).

(C) The total plasma and free saliva concentrations ($p< 0.001$, r= 0.679) and **(D)** the free concentrations in plasma and saliva were determined to confirm the associations of the free PER concentration in saliva with the total and free concentrations in plasma, and the correlations were significant ($p< 0.001$, r= 0.710).



4. Effect of CYP3A4 enzyme inducers on AED concentrations

The enzyme inducers decreased the PER concentrations in both plasma and saliva, but did not affect the correlations. Eleven of the 30 patients received the CYP3A4 inducer CBZ or OXC in combination with PER. The mean value of the total PERcorr_conc. in plasma in the inducer group was 37.41 ± 26.4 ng/ml, which was approximately 54.4% lower than in the noninducer group (82.05 ± 48.97 ng/ml, n = 19) (p = 0.012). In addition, the average free PERcorr_conc. in plasma in the inducer group was approximately 42.4% lower than in the noninducer group (p = 0.021). The average total PERcorr_conc. in saliva was decreased by 31.9% in the inducer group compared to the noninducer group, but the difference was not significant (p = 0.236). On the other hand, the free PERcorr_conc. in saliva in the inducer group was decreased by approximately 39.6% compared to in the noninducer group (p = 0.008). (**Figure 4**). The GMR showed a significant difference of less than 0.8 between the two groups for all concentrations. (**Table 5**). We calculated the partial correlation coefficient with the inducer as a control variable to consider the interaction of concomitant CYP3A4 inducers with PER. However, the correlation coefficient between the saliva and plasma concentrations did not decrease when the use of CYP3A4 inducers was considered as a control variable, indicating that the CYP3A4 inducers did not affect these correlations (**Table 6**).

Figure 4. Comparison of the PERcorr_conc. in patients treated with or without a concomitant CYP3A4 inducer.

(A) and (B) The effects of the inducer on the total and free PER concentrations in plasma were significant at $p < 0.05$. The effect of the inducer on saliva concentrations was different from the effect on the plasma concentrations. (C) The total PERcorr_conc. showed a p–value greater than 0.05, while (D) the free PERcorr_conc. showed a significant difference.

* PERcorr_conc.: corrected perampanel concentration (concentration per daily dose)

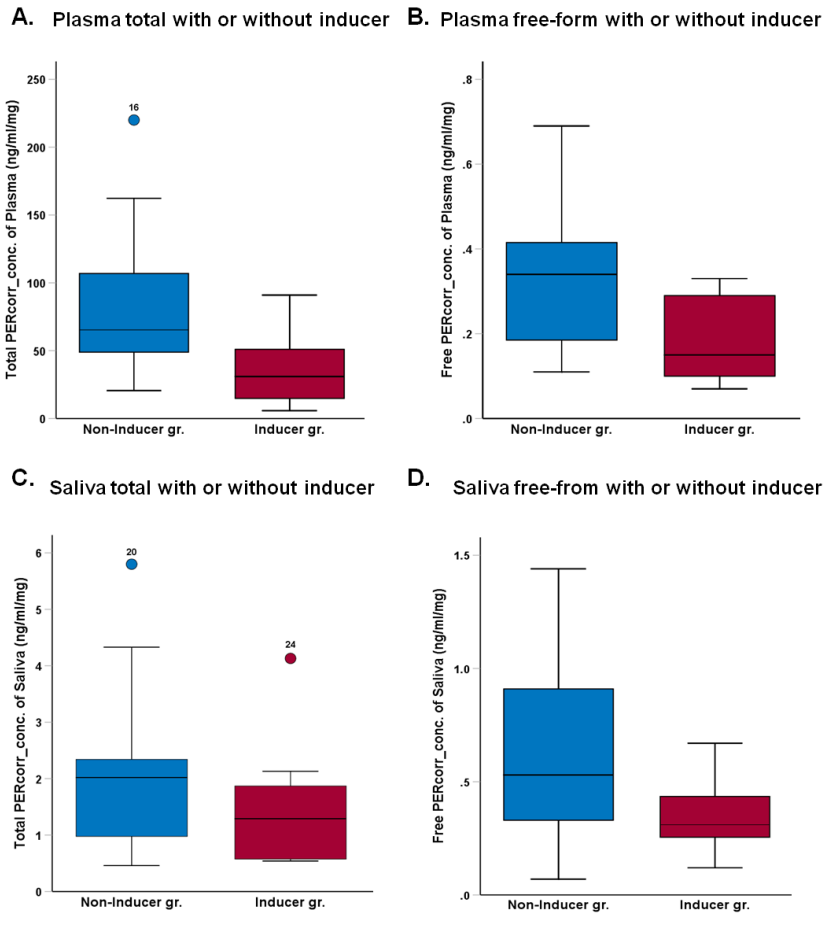


Table 5. Corrected PER concentrations in patients treated with or without a CYP 3A4 inducer.

	Plasma		Saliva	
	Total PERcorr_conc.[a]	Free PERcorr_conc.[a]	Total PERcorr_conc.[a]	Free PERcorr_conc.[a]
Mean ± SD				
Noninducer gr. (n=19)	82.05 ± 48.97	0.33 ± 0.16	2.01 ± 1.28	0.64 ± 0.37
Inducer gr. (n=11)	37.41 ± 26.4	0.19 ± 0.09	1.45 ± 1.01	0.35 ± 0.15
p value	0.012	0.021	0.236	0.008
Geometric Mean				
Noninducer gr.	69.61	0.29	1.66	0.53
Inducer gr.	27.97	0.17	1.18	0.32
Geometric Mean Ratio[b] (90% CI)	0.4 (0.26 – 0.63)	0.6 (0.42 – 0.85)	0.71 (0.46 – 1.08)	0.6 (0.41 – 0.88)

[a]Corrected PER concentration; concentration(ng/ml) per daily dose(mg/day)
[b]Inducer to noninducer GMR

Table 6. Partial correlation coefficients in patients concomitantly treated with CYP 3A4 inducers

	Plasma Total Conc.	Plasma Free Conc.	Saliva Total Conc.	Saliva Free Conc.
Plasma Total Conc.	1			
Plasma Free Conc.	0.836 → 0.838*	1		
Saliva Total Conc.	0.678 → 0.721*	0.619 → 0.633*	1	
Saliva Free Conc.	0.679 → 0.666*	0.710 → 0.703*	0.607 → 0.627*	1

*$p < 0.001$
**The correlation coefficients were calculated before and after the CYP3A4 inducer was set as a control variable

IV. Discussion

This study is the first to measure PER concentrations in matched plasma and saliva samples from patients with epilepsy. A significant correlation was observed between PER concentrations in the two specimens. In particular, the total PER concentration in saliva and the free PER concentration in plasma showed a linear correlation. In addition, concomitant administration of a CYP3A4 inducer did not affect the correlation between the plasma and saliva concentrations.

In our study, the total PER concentration in saliva correlated well with the free PER concentration in plasma. Normally, the free form of a drug in plasma is considered the active compound responsible for the pharmacological effects. However, the free concentration is more difficult to measure than the total concentration, as it requires additional procedures for protein filtration. Moreover, saliva samples are much easier to obtain than blood samples. Therefore, the observation of a correlation between the total drug concentration in the ‘easily measurable’ saliva sample and the ‘pharmacologically important’ plasma free drug concentration in plasma should be useful for understanding PER concentrations and the value of TDM. Although the absolute plasma concentrations were not able to be derived from the saliva concentrations, we propose that the therapeutic range of PER concentrations in saliva could be determined in future studies.

The free concentration of PER in saliva was higher than in plasma. The free concentration in saliva was 29% of the total concentration in plasma, whereas the free concentration in plasma was 0.45% of the total concentration in plasma. A potential explanation for this discrepancy is that the protein content in saliva is approximately 3% of the plasma protein content.[100] In addition, the different concentrations of free PER in saliva and plasma may be related to the fact that saliva is condensed and secreted rather than circulated like blood. Nevertheless, saliva is a potentially useful sample for monitoring PER concentrations, because the correlation presented above was significant.

The presence of a CYP3A4 inducer decreased the PER levels in saliva but did not affect the correlation between saliva and plasma PER levels. The interaction of PER with CYP 3A4 inducers has been reported in many studies,[5,97,101,102] and our results also showed a significant reduction in PER concentrations (PERcorr_conc.) in patients taking enzyme–inducing AEDs. The GMR was less than 0.8 in both plasma and saliva, indicating that CYP3A4 inducers significantly altered the concentration of PER. In addition, the partial correlation analysis correcting for the effect of the inducer as a control variable produced a high correlation, as shown in Table 6, indicating a genuine correlation between plasma and saliva concentrations. In other words, the presence of a CYP3A4 inducer did not affect the correlation between plasma and saliva concentrations. VPA is classified as a CYP 3A4 enzyme inhibitor. As shown in the study by Contin et al.,[102] VPA exerts a considerable effect on PER, while Patsalos et al.[96] did not observe a substantial effect. In addition,

because the number of patients taking VPA in our study was small (3 of 30), our study did not consider the effects of VPA as an enzyme inhibitor. We additionally assessed the GMR ratio after excluding the 3 patients who were taking VPA (patient numbers 2, 9, and 24), and the results of this supplementary analysis are provided below. We confirmed that the presence of VPA did not affect the main findings of the current study.

	Geometric mean		Geometric mean ratio
	Inducer	Non–inducer	
Plasma total conc.	27.97 → 30.49	69.61 → 69.62	0.4 → 0.44
Plasma free conc.	0.17 → 0.18	0.29 → 0.28	0.6 → 0.64
Saliva total conc.	1.18 → 1.21	1.66 → 1.66	0.71 → 0.73
Saliva free conc.	0.32 → 0.33	0.53 → 0.53	0.6 → 0.62

For a further expansion of the discussion, our data (although the number of subjects was not enough) enabled us to infer the reference range of saliva PER concentration. The reference range of the plasma total concentration was 180 – 980 ng/ml in a previous study.[19] In addition, our total level in saliva was approximately 3% of the plasma total PER concentration. Based on these findings, the putative reference range of saliva is approximately 5.4 – 29.4 ng/ml. Of course, further studies of a larger number of subjects are needed to confirm the reliability and reproducibility of the data and to verify the consistency of the values in each individual.

Measurements of AED concentrations immediately after a seizure or after the appearance of suspected side effects may provide a substantial diagnostic benefit. A study aiming to confirm the drug concentration by

obtaining saliva immediately after the occurrence of a seizure or side effects should be conducted in the future. When the event occurs outside the therapeutic range of the AED concentration, pharmacokinetic actions should be suspected. However, when the seizure occurs within the therapeutic range of the AED, pharmacodynamic properties should be considered to underlie the treatment failure. Numerous TDM studies have been performed on saliva since it is able to be obtained immediately after a seizure occurring at home by the patients or caregivers.[103–108] Significant associations between blood and saliva concentrations have been suggested in many studies (Table. 1), but no data have been obtained from patients taking PER.

We have revealed the potential to substitute plasma with saliva for TDM of PER. We did not use any specialized collection devices in our study to maximize the simplicity of sample collection. Inexpensive, readily available 15ml BD Falcon™ polypropylene tubes (BD Biosciences, USA) were used to collect a target volume 2 ml of saliva, an amount for which the patient would not feel burdened to produce. The total time required for saliva collection was less than 3 minutes. However, the maximum duration that saliva samples can be stored at ambient temperature or in a refrigerator without affecting the results should be investigated, and studies would be then be able to be performed in real clinical settings. Saliva should be collected for a precise measurement of the PER concentration during the event by the patient or patient's guardian immediately after the occurrence of a seizure or suspected toxic event prior to arriving at the hospital.

This study has some limitations. As stated above, only a small number of patients was included in the current study. However, the saliva concentration of PER in patients with epilepsy was assessed for the first time in a real clinical setting, which is valuable. The therapeutic range of PER concentrations in saliva is presently unknown and studies examining a larger number of patients should be performed to attempt to validate a therapeutic range for PER concentrations in saliva.

V. Conclusions

Based on these data, saliva is a suitable substitute for plasma in the TDM of PER. The PER concentration in saliva was significantly correlated with the concentration in plasma, and this correlation was not affected by the concomitant administration of a CYP3A4 inducer. Subsequent studies should be conducted in the future to assess the clinical utility of TDM of PER concentrations in saliva samples from patients with epilepsy.

VI. Contributions

Do–Yong Kim contributed to the study concept and design; Do–Yong Kim and KaHeon Song acquired the data; Do–Yong Kim, Jangsup Moon, Yong–Won Shin, SeungHwan Lee, Kyung–Sang Yu, In–Jin Jang, and KaHeon Song analyzed and interpreted the data; Do–Yong Kim and Jangsup Moon drafted the manuscript and designed the figures; Yong-Won Shin, Soon-Tae Lee, Keun–Hwa Jung, Kyung–Il Park, Ki–Young Jung, Manho Kim, SeungHwan Lee, Kyung–Sang Yu, In–Jin Jang, Kon Chu, and SangKun Lee critically revised the manuscript for important intellectual content; and Kon Chu and SangKun Lee supervised the study.

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요약 (국문초록)

서론: 비교적 좁은 치료지수(therapeutic index)를 가지고 주로 장기 복용을 요하는 항 뇌전증제(AED)에서의 치료적 약물모니터링(TDM)은 뇌전증 환자의 약물 관리를 최적화하는 데 도움을 준다. 특히, 경련, 발작이나 약물 부작용 등이 발생한 직후의 혈중 약물 농도의 측정은 그 원인이 약동학적 혹은 약력학적 문제임을 판별하는 데에 중요한 단서가 된다. 대부분의 의료기관에서 TDM 은 주로 혈액을 통해 검사되고 있는데, 이는 침습적이고, 반드시 의료기관 내에서 실시되어야 하는 등의 접근성 부분에서 한계를 가진다. 이와 달리 타액 채취는 비침습적이며 자발적인 채취가 가능하다는 등의 장점을 가지고 있어, AED TDM 의 대상으로서의 타액 연구는 많이 보고되어 왔다. 본 연구는 아직까지 연구가 전무한 페람파넬 TDM 에서의 타액이 혈액의 대체제가 될 수 있는지 평가하고자 한다.

방법: 본 연구는 2018 년 8 월부터 2019 년 3 월까지 서울대학교 병원 신경과 외래를 방문한 페람파넬을 복용 중인 뇌전증 환자를 대상으로 하였다. 혈액과 타액을 동 시간대에 채취하여 페람파넬의 혈중 총 농도 및 유리 농도를 LC–MS/MS 와 HPLC 를 통해 측정하고 상관도를 관찰했다. 또한, 타액과 혈장의 혈중 농도에 대한 상관도가 CYP3A4 inducer 에 의해 유의한 간섭을 받는지 확인했다.

결과: 총 30 명이 등록되었으며, 연령 범위는 16–60 세, 10 명은 여성 (33%) 이였다. 페람파넬의 복용용량은 2–12 mg (평균 6mg) 였다.

페람파넬의 총 및 유리 혈중농도는 각각, 혈장: 343.02 (46.6–818.0), 1.53 (0.51–2.92) ng/ml, 타액: 9.74 (2.21–33.0), 2.83 (1.01–6.8) ng/ml 였다. 타액의 총 혈중농도와 혈장의 총 및 유리 혈중농도와의 상관계수는 각각, r=0.678, r=0.619 였고, 모두 p<0.001 수준에서 유의함을 보였다. 또한, CYP3A4 inducer 는 이러한 타액과 혈장의 상관도에 유의한 영향을 주지 않았다.

결론: 페람파넬의 TDM 에서 타액은 혈장과 유의한 상관도를 보였으며, 이 상관도에 CYP3A4 inducer 는 어떠한 영향도 미치지 못했다. 본 연구의 결과는 최초로 페람파넬의 TDM 에서 타액이 혈액의 대체제로서의 가능성이 있음을 입증하였다.

주요어: 치료적 약물모니터링, 페람파넬, 타액, 혈액, 상관성 분석

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