Received: 12 May 2020 | Revised: 27 May 2020 | Accepted: 27 May 2020

DOI: 10.1111/epi.16584

Epilepsia®

BRIEF COMMUNICATION

Relationship between saliva and plasma rufinamide concentrations in patients with epilepsy

Valentina Franco[1,2] | **Giuliana Gatti**[1] | **Iolanda Mazzucchelli**[1] | **Roberto Marchiselli**[1] | **Cinzia Fattore**[2] | **Paola Rota**[3] | **Carlo Andrea Galimberti**[2] | **Giuseppe Capovilla**[4] | **Francesca Beccaria**[4] | **Valentina De Giorgis**[2] | **Cecilie Johannessen Landmark**[5,6] | **Emilio Perucca**[1,2]

[1]Division of Clinical and Experimental Pharmacology, Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy

[2]IRCCS, Mondino Foundation, Pavia, Italy

[3]Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, Italy

[4]Epilepsy Center, C. Poma Hospital, Mantua, Italy

[5]Program for Pharmacy, Faculty of Health Sciences, Oslo Metropolitan University, Oslo, Norway

[6]Section for Clinical Pharmacology, Department of Pharmacology, National Center for Epilepsy, Oslo University Hospital, Oslo, Norway

Correspondence
Emilio Perucca, Division of Clinical and Experimental Pharmacology, Department of Internal Medicine and Therapeutics, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy.
Email: perucca@unipv.it

Funding information
IRCCS Mondino Foundation (Ricerca Corrente 2009-2011).

Abstract

The assay of saliva samples provides a valuable alternative to the use of blood samples for therapeutic drug monitoring (TDM), at least for certain categories of patients. To determine the feasibility of using saliva sampling for the TDM of rufinamide, we compared rufinamide concentrations in paired samples of saliva and plasma collected from 26 patients with epilepsy at steady state. Within-patient relationships between plasma rufinamide concentrations and dose, and the influence of comedication were also investigated. Assay results in the two tested fluids showed a good correlation ($r^2 = .78$, $P < .0001$), but concentrations in saliva were moderately lower than those in plasma (mean saliva to plasma ratio = 0.7 ± 0.2). In eight patients evaluated at three different dose levels, plasma rufinamide concentrations increased linearly with increasing dose. Patients receiving valproic acid comedication had higher dose-normalized plasma rufinamide levels than patients comedicated with drugs devoid of strong enzyme-inducing or enzyme-inhibiting activity. Overall, these findings indicate that use of saliva represents a feasible option for the application of TDM in patients treated with rufinamide. Because rufinamide concentrations are lower in saliva than in plasma, a correction factor is needed if measurements made in saliva are used as a surrogate for plasma concentrations.

KEYWORDS

drug assay, plasma, rufinamide, saliva, therapeutic drug monitoring

1 | INTRODUCTION

Rufinamide is a second-generation antiseizure medication (ASM) approved in the USA, Europe, and elsewhere for the adjunctive treatment of seizures associated with Lennox-Gastaut syndrome in patients 1 year of age and older.[1,2] Pharmacokinetic studies have shown that there is a wide variability in plasma rufinamide concentrations among patients receiving the same dose, due to intersubject differences in extent of gastrointestinal absorption and

metabolic clearance.[3] Evidence has been provided that improvement in seizure control and occurrence of adverse effects in patients treated with rufinamide correlate positively with the plasma concentration of the drug, suggesting that therapeutic drug monitoring (TDM) may aid in individualizing rufinamide dosing.[3] Repeated blood sampling for TDM, however, can be a cause for discomfort, particularly for children, and the possibility of using a non-invasively accessible biological fluid such as saliva could be advantageous.

For many ASMs, the drug concentration in saliva correlates strongly with the unbound (pharmacologically active) concentration in plasma, which allows use of salivary samples instead of plasma for TDM purposes.[4–7] Rufinamide is a lipophilic molecule that binds to a modest extent (26%-34%) to plasma proteins,[3] and preliminary data from our laboratory using samples from a single patient evaluated at three different dose levels suggest that there is a close correlation between salivary and plasma rufinamide concentrations.[8] The purpose of the present study is to formally investigate the relationship between the concentration of rufinamide in plasma and in saliva in patients with epilepsy, to determine the potential applicability to TDM of salivary samples. The study also provided the opportunity to re-evaluate the relationship between plasma rufinamide concentrations and prescribed daily dose, and the influence of concomitant medications on rufinamide pharmacokinetics.

2 | MATERIALS AND METHODS

This was a prospective study in patients with epilepsy stabilized on treatment with rufinamide at the C. Mondino National Neurological Institute in Pavia and at the C. Poma Hospital in Mantua. Eligibility criteria included (1) a diagnosis of epilepsy; (2) receiving rufinamide as part of routine clinical management according to authorized indications, which in Italy include the add-on treatment of seizures associated with Lennox-Gastaut syndrome in patients 1 year of age and older, as well as seizures associated with other epileptic encephalopathies in children older than 4 years; (3) being at steady state, that is, at least 48 hours after the last rufinamide dose change (at least 96 hours for patients comedicated with valproic acid); and (4) written informed consent. No a priori sample size calculations were made, as this was an exploratory study. The study protocol was approved by the ethics committees of participating centers.

From each patient, one blood (5-6 mL) and one saliva sample (2 mL) were collected simultaneously in the morning, prior to the first morning dose of rufinamide and between 12 and 16 hours after the last dose. Patients were asked to expectorate unstimulated saliva into untreated polypropylene tubes. Whenever possible, samples were collected at more than one dose level in each patient. The blood was transferred to an EDTA tube without gel separator and centrifuged within 1 hour (1200 × *g* for 15 minutes), and plasma and saliva samples were stored at −20°C until assay. The concentration of rufinamide in plasma and saliva was determined by using validated high-performance liquid chromatography assay with ultraviolet light detection according to Mazzucchelli et al.[8] The assay has a lower limit of quantitation of 0.25 μg/mL (1.05 μmol·L^{-1}), which is adequate to measure total and unbound plasma rufinamide concentrations in patients treated with doses within the clinically used range.

Results are presented as means ± SD unless otherwise specified. The normal distribution of the assay results was evaluated by Shapiro-Wilk test, and the linearity of the relationship between plasma and saliva measurements samples by Cusum test. Correlations between paired plasma and saliva assay results were assessed by Pearson regression analysis. The level of agreement between plasma and saliva concentrations was also evaluated by Passing-Bablok regression analysis, with calculation of 95% confidence intervals for the slope and intercept of the regression line. For all tests, levels of statistical significance were set at 5%. Statistical analyses were done using Stata Statistical Software version 14 (StataCorp).

3 | RESULTS

Twenty-eight patients were enrolled, but two had to be excluded from the analysis because they could not provide a sufficient volume of saliva. The patients with assessable paired plasma and saliva samples included 15 males and 11 females, with a median age of 16 years (range = 4-38 years) and average rufinamide doses of 24 mg/kg/d (range = 7-70 mg/kg/d; Table 1). Because 16 patients provided samples at more than one dose level, a total of 50 paired samples of plasma and saliva were evaluated.

Based on the Shapiro-Wilk test, plasma and saliva concentrations did not deviate from a normal distribution ($P > .05$). The mean rufinamide concentration in all assayed samples was 9.1 ± 3.6 μg/mL (range = 1.2-29.7 μg/mL) for plasma and 6.5 ± 3.2 μg/mL (range = 0.8-20.2 μg/mL) for saliva. The mean saliva to plasma concentration ratio was 0.7 ± 0.2 (range = 0.4-1.1).

Application of the Cusum test to the relationship between plasma and saliva assay results revealed no significant deviation from linearity ($P > .20$). Pearson correlation indicated that there was a good correlation between plasma and saliva concentrations ($r^2 = .78$, n = 26; $P < .0001$), as also confirmed by Passing-Bablok regression analysis (Figure 1). However, saliva concentrations were moderately lower than plasma concentrations, consistently with the reported saliva to plasma concentration ratio of 0.7 ± 0.2.

TABLE 1 Details of the 26 patients included in the analysis

Patient number	Age, y	Sex	Weight, kg	Rufinamide doses assessed, mg/kg/d	Plasma rufinamide concentration, μg/mL[a]	Saliva rufinamide concentration, μg/mL[a]	Concomitant ASMs
1	7	M	23	43, 52, 70	5.7 (4.6-6.3)	3.9 (2.7-5.6)	OXC, ZNS
2	16	M	56	7, 18, 29	11.9 (6.8-16.7)	8.1 (4.0-12.6)	VPA, LTG
3	10	F	25	32, 40	4.6 (2.4-6.9)	2.7 (1.7-3.7)	CZP, ZNS, PB
4	8	M	21	10, 14, 29	4.4 (2.4-5.9)	2.2 (1.5-2.9)	NZP, VPA, LTG
5	18	F	35	11, 46	3.5 (1.2-5.7)	2.5 (0.8-4.2)	OXC, TPM
6	8	M	20	20, 30	9.2 (6.3-12.2)	8.1 (6.3-9.8)	VPA, LTG
7	20	M	58	7, 28	5.8 (1.3-10.4)	3.3 (0.9-5.7)	FBM, CZP, PB, TPM
8	8	M	27	30, 37	9.8 (9.0-10.6)	5.4 (4.7-6.1)	ZNS
9	21	M	35	11, 17, 34	9.7 (6.3-12.3)	6.9 (4.4-9.2)	VPA, ETS, LEV
10	17	M	48	17, 25	9.9 (7.2-12.5)	5.5 (4.2-6.7)	VPA, CZP, ZNS
11	14	F	81	15, 25	15.8 (14.9-16.7)	13.1 (12.0-14.3)	NZP, VPA, VGB
12	7	F	26	15, 31, 38	7.7 (2.4-10.4)	4.7 (1.7-6.2)	CZP, ZNS
13	10	F	21	10, 29, 48	2.3 (1.4-3.1)	1.3 (1.0-1.5)	CLB, LEV, VGB
14	13	M	36	17, 28, 50	10.1 (4.7-14.7)	7.7 (2.8-13.5)	VPA, LEV
15	4	M	17	12, 35	7.6 (3.8-11.4)	5.9 (3.5-8.4)	VPA, TPM
16	11	F	39	10, 15, 31	14.2 (3.8-29.7)	10.9 (3.8-20.2)	VGB
17	18	M	47	34	9.2	3.7	VPA, ETS
18	10	F	35	12	9.2	9.6	VPA, TPM
19	6	F	15	27	6.8	6.5	TPM
20	9	M	28	14	16.2	9.9	FBM, VPA, OXC
21	17	M	46	13	6.4	7.0	FBM, VPA, ZNS
22	11	F	33	12	11.1	8.0	VPA
23	15	F	72	22	7.5	4.2	LTG, LEV
24	38	M	45	27	10.8	9.3	FBM, CLB, NZP
25	24	F	55	15	13.2	12.4	TPM
26	7	M	22	27	9.6	7.3	VPA, LTG, ZNS, DZP

Note: Conversion factor from μg/mL to μmol/L: 4.198.

Abbreviations: ASMs, antiseizure medications; CLB, clobazam, CZP, clonazepam; DZP, diazepam; ETS, ethosuximide; F, female; FBM, felbamate; LEV, levetiracetam; LTG, lamotrigine; M, male; NZP, nitrazepam, OXC, oxcarbazepine; PB, phenobarbital; TPM, topiramate; VGB, vigabatrin; VPA, valproate; ZNS, zonisamide.

[a]Mean and range.

The within-patient relationship between plasma rufinamide concentration and daily dose was evaluated for the eight patients who could be assessed at three different dose levels. The concentrations measured at the lowest, intermediate, and highest dose assessed for each patient were averaged and plotted against mean corresponding doses. As illustrated in Figure 2, the relationship was linear across the dose range tested.

The influence of concomitant medications on plasma rufinamide concentrations was also investigated. In view of the linear relationship between plasma rufinamide concentrations and dose within patients, plasma concentrations (μg/mL) were dose-normalized by dividing them by the prescribed daily dose (mg/kg). Dose-normalized concentrations were then compared between patients comedicated with valproic acid, a known inhibitor of various drug-metabolizing enzymes, and patients comedicated with ASMs considered to have little or no influence on drug metabolism (benzodiazepines, ethosuximide, lamotrigine, levetiracetam, oxcarbazepine, topiramate, vigabatrin, and zonisamide). For patients with multiple samples, the mean dose-normalized concentration was used for each individual. Patients receiving felbamate, an enzyme inhibitor, and phenobarbital, an enzyme inducer, were excluded from this comparison due to their potential confounding effects. Dose-normalized plasma rufinamide concentrations (μg/mL per mg/kg) were found to be 0.55 ± 0.28 in the 14 patients comedicated with valproic acid (either alone or in combination with non–enzyme-inducers/inhibitors) compared with 0.35 ± 0.28 in the nine patients taking other ASMs but no strong enzyme inducers or inhibitors ($P < .04$, Student t test).

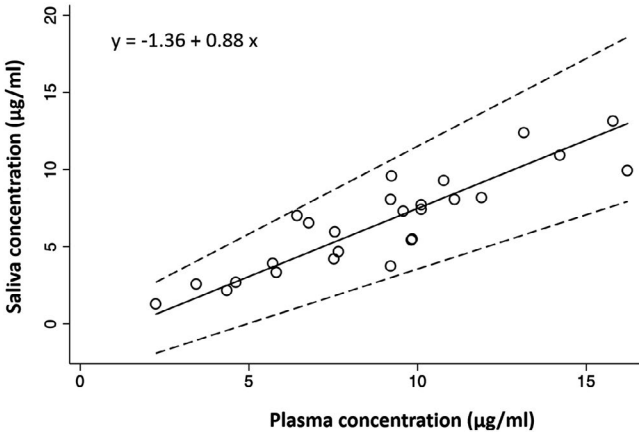

FIGURE 1 Passing-Bablok plot regression analysis illustrating the relationship between measured plasma and saliva concentrations in paired samples from 26 patients. For patients assessed at more than one dose level, mean measured plasma and saliva concentrations were used for each individual. The regression line is indicated by the continuous line. Broken lines indicate 95% confidence intervals (CI). Parameter estimates (95% CI) were 0.88 (0.70-1.13) for the slope and −1.36 (−3.5 to 0.15) for the intercept. Conversion factor from μg/mL to μmol/L: 4.198

4 | DISCUSSION

TDM, a well-established tool to improve individualization of dose of many ASMs, is usually performed on plasma or serum samples.[4] Collection of blood samples, however, can be a cause of discomfort, particularly for children and individuals with intellectual disability, and may be problematic for certain categories of patients, such as those with coagulation disorders or difficult venous access. In addition, patients must visit a clinic, as blood sampling requires intervention of a phlebotomist. Hence, there is a growing interest in the use of minimally invasive alternative sampling strategies. An attractive approach is the use of saliva drug concentrations, which for certain medications offers the added advantage of being closely correlated with the concentration of unbound, pharmacologically active drug in plasma.[5,9] A strong correlation between saliva and plasma concentration, however, is not found for all drugs, and needs to be investigated specifically for each medication.[5,9]

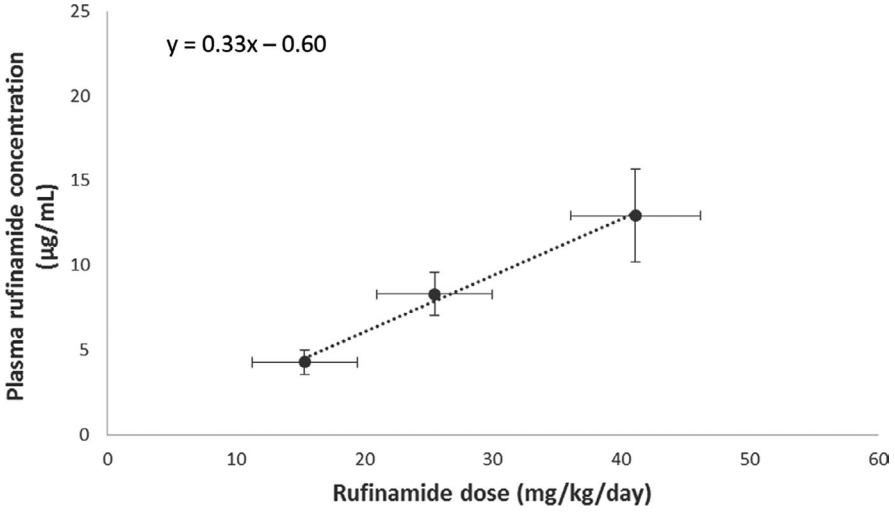

FIGURE 2 Relationship between plasma rufinamide concentration and prescribed daily dose in eight patients assessed at three different dose levels. Plasma concentrations measured at the lowest, intermediate, and highest dose in each patient were averaged, and plotted against corresponding mean doses. Bars indicate standard errors. The equation of the line refers to Pearson linear regression

Prior to the present study, information on the concentration of rufinamide in saliva was limited to data from a single individual.[8] We extended those observations and demonstrated that saliva rufinamide concentrations in predose morning samples collected at steady state in patients with epilepsy correlate well with plasma concentrations. Saliva concentrations, however, were moderately lower than plasma concentration (about 70% on average), indicating that a correction factor needs to be applied if salivary concentration is used as a surrogate for plasma concentration in a TDM setting. If a correction factor based on the mean saliva/plasma concentration ratio is applied, users should be aware of potential inaccuracy resulting from variability in the ratio across samples (range = 0.4-1.1). Because rufinamide binding to plasma proteins is about 70%,[3,10] it is tempting to speculate that saliva concentrations could reflect the concentration of unbound (pharmacologically active) drug more accurately than total plasma concentrations. This hypothesis requires formal testing in future studies incorporating measurements of unbound plasma rufinamide concentrations.

Early pharmacokinetic studies indicated that, in adults, plasma rufinamide concentrations increase in an approximately linear manner up to a dose of 1600 mg/d, and that at higher doses the increase in plasma concentrations is less than proportional because of reduced extent of absorption.[3] A less than proportional increase in plasma rufinamide levels with increasing dose has also been reported in more recent studies,[11,12] but there have also been reports indicative of dose-independent (linear) pharmacokinetics.[11] Our observations made in eight patients assessed at three different dose levels revealed a linear increase in plasma concentrations with increasing doses within the explored dose range, although we cannot exclude a deviation from linearity at higher doses. With respect to other findings from the present study, we confirmed that patients comedicated with valproic acid have higher plasma rufinamide concentrations than patients receiving ASMs devoid of strong enzyme-inducing or enzyme-inhibiting activity.[3,11,13] Rufinamide is metabolized primarily by enzymatic hydrolysis,[3] and the elevating effect of valproic acid on plasma rufinamide concentration is most likely explained by inhibition of human carboxylesterase type 1, as documented by in vitro studies.[14]

In conclusion, we have provided evidence that the concentration of rufinamide in saliva correlates well with its concentration in plasma. The agreement between assay values may be sufficiently high to permit use of saliva samples for TDM purposes in patients with epilepsy treated with rufinamide. Because saliva concentrations are moderately lower than plasma concentrations, however, a correction factor is needed if saliva concentrations are used as a surrogate for plasma concentrations. As rufinamide is used mostly in pediatric patients, the use of saliva samples could be especially convenient in this population. As our measurements were made in predose morning samples, we did not investigate the possibility of saliva to plasma rufinamide concentration ratios being subject to variation during a dosing interval. Utilization of postdose saliva samples for TDM purposes, however, is not advisable, particularly when using suspension formulations that may result in saliva being contaminated by drug residues in the mouth.

ACKNOWLEDGMENTS

This study was supported by a grant from the IRCCS Mondino Foundation (Ricerca Corrente 2009-2011). Dr Guido Fedele is gratefully acknowledged for advice on the statistical analysis of the data.

CONFLICT OF INTEREST

F.B. received consultancy fees from Eisai and GW Pharma. V.F. received consultancy fees from GW Pharma. C.A.G. received speaker's or consultancy fees from Bial-Portela & Ca, Eisai, Lusofarmaco, Sandoz-Novartis Farma, Sanofi, and UCB Pharma. E.P. received speaker's or consultancy fees from Amicus Therapeutics, Arvelle, Biogen, Eisai, GW Pharma, Intas Pharmaceuticals, Laboratorios Bagò, Sanofi, Sun Pharma, UCB Pharma, and Xenon Pharma. C.J.L. received speaker's fees from GW Pharma, Eisai, and Labor Krone. The remaining authors have no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

AUTHOR CONTRIBUTIONS

V.F. and E.P. were involved in the concept and design of the study. F.B., G.C., V.D.G., and C.A.G. contributed to patient enrolment and collection of samples. V.F., G.G., R.M., and I.M. performed the assay. C.F., C.J.L., and P.R. performed the statistical analysis. All authors participated in the interpretation of the data, critical review of the manuscript, and the decision to submit for publication.

ORCID

Valentina Franco https://orcid.org/0000-0003-4736-4437
Cecilie Johannessen Landmark https://orcid.org/0000-0002-0877-8912
Emilio Perucca https://orcid.org/0000-0001-8703-223X

REFERENCES

1. Banzel (rufinamide). Full prescribing information. Woodcliff Lake, NJ: Eisai. Last revised February 2015. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/021911s012lbl.pdf. Accessed April 16, 2020.
2. Inovelon (rufinamide). Summary of product characteristics. Hatfield, UK: Eisai. Last revised July 2019. Available at: https://www.medicines.org.uk/emc/product/410/smpc . Accessed April 16, 2020.

3. Perucca E, Cloyd J, Critchley D, Fuseau E. Rufinamide: clinical pharmacokinetics and concentration-response relationships in patients with epilepsy. Epilepsia. 2008;49:1123–41.
4. Patsalos PN, Berry DJ, Bourgeois BFD, et al. Antiepileptic drugs—best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia. 2008;49:1239–76.
5. Patsalos PN, Berry DJ. Therapeutic drug monitoring of antiepileptic drugs by use of saliva. Ther Drug Monit. 2013;35:4–29.
6. Kim DY, Moon J, Shin YW, et al. Usefulness of saliva for perampanel therapeutic drug monitoring. Epilepsia. 2020;1–9. doi: 10.1111/epi.16513.
7. Brandt C, Bien CG, Helmer R, May TW. Assessment of the correlations of lacosamide concentrations in saliva and serum in patients with epilepsy. Epilepsia. 2018;59:e34–9.
8. Mazzucchelli I, Rapetti M, Fattore C, et al. Development and validation of an HPLC-UV detection assay for the determination of rufinamide in human plasma and saliva. Anal Bioanal Chem. 2011;401:1013–21.
9. Patsalos PN, Zugman M, Lake C, et al. Serum protein binding of 25 antiepileptic drugs in a routine clinical setting: a comparison of free non-protein-bound concentrations. Epilepsia. 2017;58:1234–43.
10. Liu H, Delgado MR. Therapeutic drug concentration monitoring using saliva samples. Focus on anticonvulsants. Clin Pharmacokinet. 1999;36:453–70.
11. May TW, Boor R, Rambeck B, et al. Serum concentrations of rufinamide in children and adults with epilepsy: the influence of dose, age, and comedication. Ther Drug Monit. 2011;33:214–21.
12. Xu M, Ni Y, Zhou Y, et al. Pharmacokinetics and tolerability of rufinamide following single and multiple oral doses and effect of food on pharmacokinetics in healthy Chinese subjects. Eur J Drug Metab Pharmacokinet. 2016;41:541–8.
13. Arzimanoglou A, Ferreira JA, Satlin A, et al. Safety and pharmacokinetic profile of rufinamide in pediatric patients aged less than 4 years with Lennox-Gastaut syndrome: an interim analysis from a multicenter, randomized, active-controlled, open-label study. Eur J Paediatr Neurol. 2016;20:393–402.
14. Williams ET, Carlson JE, Lai WG, et al. Investigation of the metabolism of rufinamide and its interaction with valproate. Drug Metab Lett. 2011;5:280–9.

How to cite this article: Franco V, Gatti G, Mazzucchelli I, et al. Relationship between saliva and plasma rufinamide concentrations in patients with epilepsy. *Epilepsia*. 2020;00:1–6. https://doi.org/10.1111/epi.16584