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Semisolid enteral nutrients alter the pharmacokinetics of orally administered levetiracetam in rats

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Enteral nutrients (ENs) affect the plasma drug concentration of orally co-administered drugs, particularly those of antiepileptic drugs, such as phenytoin and carbamazepine. However, few studies have reported the interactions of levetiracetam (LEV), an upcoming antiepileptic drug, with ENs. In this study we aimed to investigate the pharmacokinetics of LEV in 55 rats after oral co-administration of LEV with liquid or semisolid ENs. Compared with the control group, co-administration with Terumeal® Soft significantly decreased the plasma LEV concentration at 0.5, 1, and 2 h and area under the plasma concentration–time curve from 0 to 3 h ($AUC_{0\rightarrow3h}$) ($P < 0.01$). However, the $AUC_{0\rightarrow3h}$ of LEV remained unchanged following the administration of Terumeal® Soft 2 h after the initial LEV administration. Moreover, co-administration with semisolid Racol® NF delayed the absorption of LEV without decreasing the $AUC_{0\rightarrow3h}$, whereas liquid Racol® NF did not alter LEV pharmacokinetics. Thus, co-administration of LEV with Terumeal® Soft reduced the absorption of LEV from the gastrointestinal tract, which was prevented by administering Terumeal® Soft 2 h after LEV administration. Semisolid Racol® NF altered LEV pharmacokinetics without decreasing its gastrointestinal absorption. Our findings suggested that careful monitoring of the plasma LEV levels is necessary when co-administering LEV with Terumeal® Soft, semisolid Racol® NF, or any other semisolid ENs, to prevent the inadvertent effects of the interaction between LEV and ENs.

1. Introduction

Enteral nutrition (EN) is essential for individuals who have a functioning gastrointestinal tract but are unable to maintain adequate oral nutritional intake (Barrett et al. 2015). EN is physiologically more natural than parenteral nutrition and helps maintain gut integrity, supporting immune function and protecting against gut atrophy (Doley 2022). However, EN affects the absorption and resultant plasma concentration of administered drugs, particularly antiepileptic drugs (Wohlt et al. 2009: Mason 2010: Boullata and Hudson 2012; Chan 2013; Ferreira Silva et al. 2014). In our previous study we showed that – with F2α®, a liquid EN, decreased the gastrointestinal absorption of phenytoin when orally administered to rats; however, this decrease was not observed when the administration interval between phenytoin and ENs was 2 h (Urashima et al. 2019). Similarly, co-administration of carbamazepine with Ensure Liquid® decreased the gastrointestinal absorption of carbamazepine, which was effectively alleviated when a 2 h interval was used (Urashima et al. 2022). These results indicated that different antiepileptic drugs are affected by different ENs; therefore, changes in the pharmacokinetics of drugs administered in various combinations with ENs need to be investigated. In addition to the classic liquid type ENs, semisolid types have been widely used recently to prevent complications and reflux esophagitis (Toh Yoon et al. 2016; Maruyama et al. 2018; Kokura et al. 2020). As semisolid ENs were reported to decrease carbamazepine absorption, which was not observed with liquid type ENs (Nagai et al. 2019b), the form of ENs should also be considered when used in combination with drugs to avoid changes in drug pharmacokinetics.

Levetiracetam (LEV) is an antiepileptic drug commonly used as an adjunct therapy for the treatment of patients with partial seizures with or without secondary generalization that are refractory to other established first-line antiepileptic drugs (AEDs) (Cereghino et al. 2000), which is efficacious in patients with generalized epilepsy (Betts et al. 2000). The efficacy of LEV is comparable to that of fosphenytoin, phenytoin, and valproate (Glauser et al. 2016; Gujjar et al. 2017; Nakamura et al. 2017). LEV shows a clean pharmacokinetic profile, almost complete absorption from the small intestine, nonsignificant plasma protein-binding, negligible cytochrome P450 metabolism, almost total excretion by the kidneys, and a good correlation between creatinine and LEV clearance (Patsalos 2000). However, owing to the required strict control of the blood levels of LEV to ensure its efficacy and prevent adverse effects, the therapeutic drug monitoring of LEV is required (Patsalos et al. 2018). Susracal®, a liquid EN, and semisolid Racol® NF do not affect LEV pharmacokinetics (Fay et al. 2005, Nagai et al. 2021). However, as phenytoin and carbamazepine are influenced by different ENs, LEV might also be affected by ENs other than those previously reported. Moreover, many other types of ENs are in use globally, thus it is necessary to investigate the pharmacokinetics of LEV following co-administration with various types of liquid or semisolid ENs for ensuring the safe concomitant use of LEV and ENs.

This study aimed to investigate the changes in LEV pharmacokinetics following oral co-administration of LEV and liquid or semisolid ENs in rats. Our study is the first to report on changes in the pharmacokinetics of LEV when co-administered with various ENs.

2. Investigations and results

2.1. Pharmacokinetics of LEV orally co-administered with liquid or semisolid ENs in rats

LEV (75 mg/kg) was orally administered to rats in combination with either 4 mL/kg distilled water (control group), liquid EN (F2α®, Racol® NF, Ensure Liquid®, or Renalen® LP), semisolid EN (OS-1® Jelly, Racol® NF, or Terumeal® Soft), or solid food (Rodent Diet®). Plasma LEV concentrations and pharmacokinetic parameters after co-administration with liquid EN or solid food are shown in Fig. 1A and Table 1, whereas those after co-administration with semisolid EN are shown in Fig, 1B and Table 2. We observed that plasma LEV concentrations were unaltered among all liquid EN and solid food groups (Fig. 1A and Table 1). In contrast, we detected that plasma LEV concentrations 0.25 and 0.5 h after co-administration with semisolid Racol® NF, and 0.25, 0.5, 1, and 2 h after co-administration with Terumeal® Soft were significantly decreased compared with those in the control group (Fig. 1B). Interestingly, compared with the control and OS-1® Jelly groups, co-administration with Terumeal® Soft significantly reduced the LEV area under the plasma concentration–time curve from 0 to 3 h ($AUC_{0\rightarrow3h}$). In addition, we observed a significantly decreased maximum plasma concentration (C_{max}) of LEV in the Terumeal® Soft group compared with that in the control group (Table 2). However, we did not detect any differences in the plasma LEV concentrations and parameters between Terumeal® separate group and the control. We also found that semisolid Racol® NF tended to decrease the $C_{max,}$ and prolong the time to reach C_{max} (T_{max}) and mean residence time (MRT) of LEV; although the effect was not statistically significant. Whereas, co-administration with OS-1® jelly did not affect the pharmacokinetics of LEV.

2.2. Acidity and viscosity of each transport sample

We evaluated the relationship between the acidity or viscosity of each EN and the effect to LEV pharmacokinetics. We found that all liquid ENs showed a weakly acidic pH of approximately 6.0, and no differences were observed among liquid ENs. Regarding semisolid ENs, we observed that OS-1® Jelly and Terumeal® Soft showed an acidic pH of approximately 3.8, whereas semisolid Racol® NF showed a pH of 6.14 ± 0.03. The viscosities of liquid ENs were as low as 4–5 mPa·s. In the case of semisolid ENs, we detected that the viscosity of OS-1® Jelly was 50.3 ± 0.4 mPa·s; however semisolid Racol® NF and Terumeal® Soft showed high viscosity values of approximately 8000 mPa·s (Table 3).

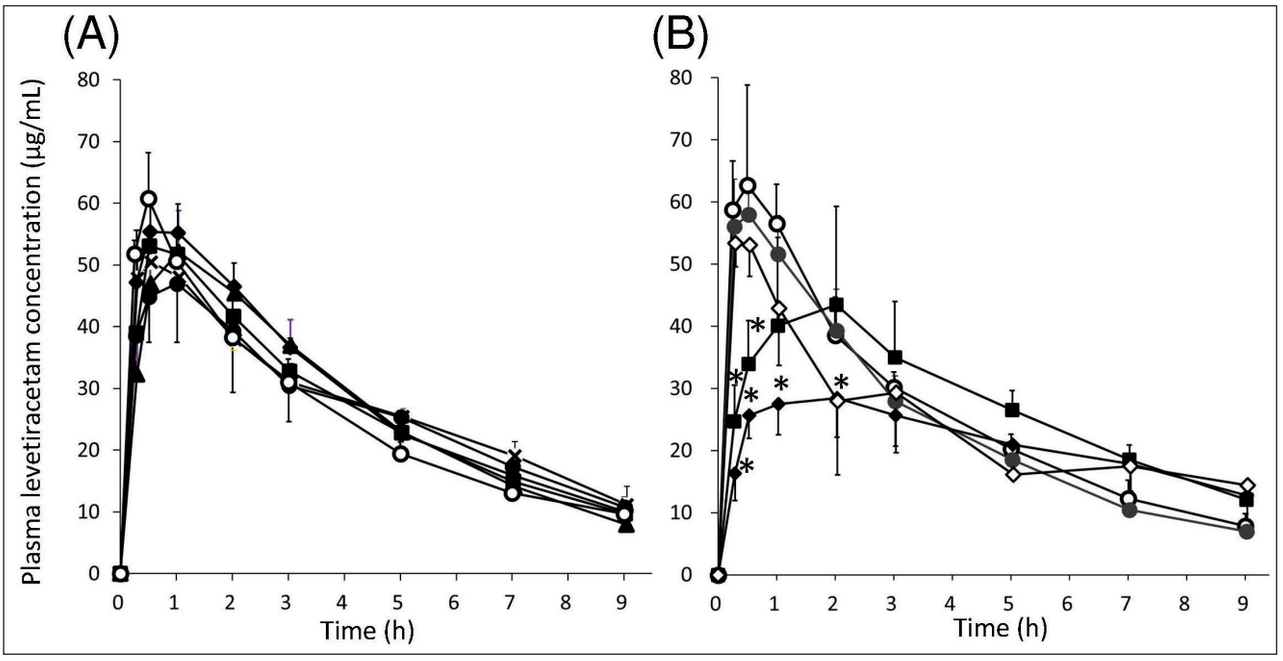

Fig. 1: (A) Levetiracetam (75 mg/kg) was orally co-administered with either distilled water, solid food or a liquid enteral formulation. ○; distilled water (control), ●; F2α® group, ■; liquid Racol® NF group, ♦; Ensure liquid® group, ▲; Renalen® LP group. , and ×; solid food group. Data are shown as the mean ± S.D. (n = 4–6). Statistical analyses were performed using the Tukey's multiple comparison test. (B) Levetiracetam (75 mg/kg) was orally co-administered with either distilled water or a semisolid enteral formulation ○; distilled water (control), ●; OS-1 Jelly® group, ■; semisolid Racol ® NF group, ♦; Termeal soft ® group and ◊; Termeal soft ® 2 h after initial LEV administration (Termeal soft ® separate group). Data are shown as the mean ± S.D. (n = 4). * $P < 0.05$, compared with control group, statistical analyses were performed using the Tukey's multiple comparison test.

Table 1: Pharmacokinetic parameters of levetiracetam after oral coadministration with liquid enteral formulations in rats

	Control	F2α®	Liquid Racol® NF	Ensure liquid®	Renalen® LP	Solid food
AUC0→3h (μg/h/mL)	132.03 ± 14.40	122.49 ± 20.23	128.56 ± 8.78	138.79 ± 11.86	128.67 ± 9.14	116.69. ± 11.58
Cmax (μg/mL)	61.73 ± 6.21	48.66 ± 7.95	54.46 ± 3.61	59.00 ± 5.51	52.05 ± 7.39	50.21 ± 4.85
Tmax (h)	0.45 ± 0.11	0.75 ± 0.29	0.70 ± 0.27	0.75 ± 0.29	1.00 ± 6.78	0.60 ± 0.22
T1/2	3.31 ± 0.78	4.23 ± 1.04	3.32 ± 0.41	3.37 ± 0.91	2.74 ± 0.26	4.62 ± 1.20
MRT	4.72 ± 0.98	6.29 ± 1.45	4.88 ± 0.49	4.99 ± 1.12	4.38 ± 0.41	6.66 ± 1.42

Levetiracetam (75 mg/kg) was orally coadministered with either distilled water, an enteral formulation, or solid food: distilled water (control), F2α®, liquid Racol® NF, Ensure liquid®, Renalen® LP, or solid food. $AUC_{0\rightarrow3h}$, area under the concentration-time curve from 0 to 3 h; C_{max}, maximum plasma concentration; T_{max}, time to reach peak serum concentration; $T_{1/2}$, elimination half-life in serum; MRT, mean residence time. Data are shown as the mean ± standard deviation (SD) (n = 4–6). Statistical analyses were performed using Tukey's multiple comparison test.

3. Discussion

In this study, we investigated the changes in LEV pharmacokinetics when administered concurrently with LEV, which is known

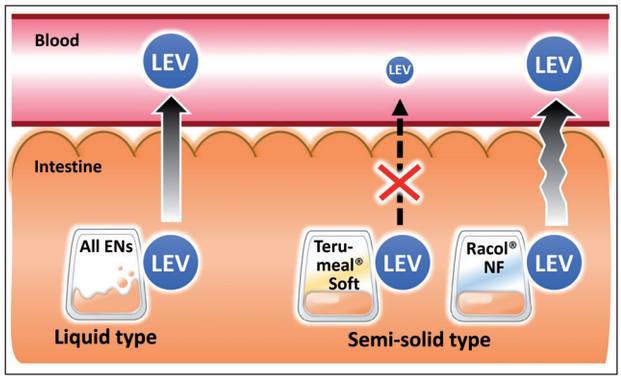

Fig. 2: Schematic representation illustrating involvement of each enteral nutrients to the LEV gastrointestinal absorption. Co-administration of LEV with all liquid ENs did not affect gastrointestinal LEV absorption. However, co-administration of LEV with Terumeal® Soft decreased the gastrointestinal absorption of LEV. Semisolid Racol® NF delayed the absorption rate of LEV with keeping total LEV absorption amount.

to have no interaction with ENs (Fay et al. 2005; Nagai et al. 2021). However, in this study, we found that some ENs affected LEV pharmacokinetics. First, we observed that liquid ENs did not alter LEV pharmacokinetics; thus, LEV can be safely co-administered with liquid ENs, in consistency with a previous study (Fay et al. 2005). Solid meals have been reported to decrease C_{max} and prolong the T_{max} of LEV in humans (2015); however, we did not observe similar finding in rats in this study.

In contrast, Terumeal® Soft significantly decreased the plasma LEV concentration at 0.25–2 h, C_{max}, and $AUC_{0\rightarrow3h}$ compared with the control (distilled water). This result suggested that Terumeal® Soft inhibits the gastrointestinal absorption of LEV. Notably, no significant changes were observed following administration of Terumeal® Soft 2 h after LEV. These results indicated that the gastrointestinal absorption of LEV was decreased following co-administration with Terumeal® Soft, and hence administration of Terumeal® Soft 2 h after LEV is an effective approach for preventing any decrease in the plasma concentration of LEV.

Semisolid Racol® NF did not affect LEV $AUC_{0\rightarrow3h}$ but decreased plasma LEV concentration at 0.25 and 0.5 h (Fig. 1B) and tended

Table 2: Pharmacokinetic parameters of levetiracetam after oral coadministration with semisolid enteral formulations in rats

	Control	OS-1® Jelly	Semisolid Racol® NF	Terumeal Soft®	Terumeal® separate
AUC0→3h (µg/h/mL)	134.21 ± 17.72	127.82 ± 15.59	109.97 ± 30.93	75.58 ± 13.54 **†	108.03 ± 22.23
Cmax (µg/mL)	64.59 ± 12.86	58.57 ± 5.86	45.37 ± 14.62	30.06 ± 3.64 **	55.26 ± 4.22
Tmax (h)	0.63 ± 0.25	0.44 ± 0.13	1.63 ± 0.75	3.00 ± 2.71	0.44 ± 0.13
T1/2	3.05 ± 0.41	4.22 ± 0.51	4.29 ± 1.39	4.30 ± 1.28	5.02 ± 1.04
MRT	4.37 ± 0.83	2.99 ± 0.42	6.69 ± 2.05	6.78 ± 1.60	7.26 ± 1.54

Levetiracetam (75 mg/kg) was orally coadministered with either distilled water, a semisolid enteral formulation, or solid food: distilled water (control), OS-1® Jelly, semisolid Racol® NF, Terumeal Soft®, and Terumeal Soft® separate groups. $AUC_{0\rightarrow3h}$, area under the concentration-time curve from 0 to 3 h; C_{max}, maximum plasma concentration; T_{max}, time to reach peak serum concentration; $T_{1/2}$, elimination half-life in serum; MRT, mean residence time. Data are shown as the mean ± SD (n = 4). * $P < 0.05$, ** $P < 0.01$ compared with control group, † $P < 0.05$, compared with OS-1® group; statistical analyses were performed using Tukey's multiple comparison test.

Table 3: Acidity and viscosity of each EN formulation

Enteral nutrition formula		Viscosity (mPaγs)	pH
	Distilled water	0.50 ± 0.00	7.79 ± 0.16
	F2α®	4.10 ± 0.99	6.72 ± 0.01
Liquid	Liquid Racol® NF	4.75 ± 0.21	6.45 ± 0.01
	Ensure liquid®	4.70 ± 0.00	6.72 ± 0.01
	Renalen® LP	5.65 ± 0.21	5.72 ± 0.06
	OS-1® Jelly	50.31 ± 0.43	3.88 ± 0.04
Semi-solid	Semisolid Racol® NF	8185.00 ± 1322.29	6.14 ± 0.03
	Terumeal® Soft	8665.00 ± 1336.43	3.81 ± 0.03

Data are shown as the mean ± SD (n = 3).

to decrease $C_{max,}$ whereas prolonged T_{max} and MRT of LEV (Table 2). This result indicated that semisolid Racol® NF might influence the antiepileptic effects of LEV. Nagai et al. reported that semisolid Racol® NF did not affect the pharmacokinetics of LEV (Nagai et al. 2021); however, LEV was continuously and slowly administered through a tube in that study. Thus, we assumed that the volume of EN administered at once in the stomach might affect the pharmacokinetics of LEV. Notably, semisolid Racol® NF altered the pharmacokinetics of LEV, whereas liquid Racol® NF did not (Fig. 1A and 1B). Both the liquid and semisolid Racol® NF contain identical nutritional components but differ only in their viscosities (Table 3) and fiber content required for semisolidification (Table 4). Nagai et al. (2019a) also reported that sodium alginate, a semisolidifying agent, decreased the plasma concentration and AUC of carbamazepine (Nagai et al. 2019a). As semisolid Racol® NF also contains sodium alginate as a semisolidifier, changes in the pharmacokinetics of LEV might also be attributed to the presence of sodium alginate.

Low-viscosity liquid EN and OS-1® did not affect plasma LEV concentrations, indicating that the high viscosity of ENs is the most important factor affecting LEV pharmacokinetics. However, although Terumeal® Soft and semisolid Racol® NF have similar viscosities (Table 3), Terumeal® Soft decreased the gastrointestinal absorption of LEV, whereas semisolid Racol® NF only delayed its absorption rate (Table 2). Of note, Terumeal® Soft differs from semisolid Racol® NF in the content of agar-agar used as a semisolidifier. Interestingly, 5 % agar-agar was reported to decrease the gastrointestinal absorption of zinc to 70 % (Kondo and Osada 1996). Although it is not possible to directly compare the effects of agar-agar and sodium alginate on the gastrointestinal absorption of LEV, we determined that formulations with different agar-agar percentages had differential effects on LEV pharmacokinetics. Moreover, despite LEV being a basic drug and as such its gastrointestinal absorption might be reduced in acidic solutions due to a decrease in the molecular type rate, we found that acidity did not affect LEV disposition. As LEV exhibits high bioavailability greater than 95 % (Howard et al. 2018), the molecular fraction in solution probably does not affect its gastrointestinal absorption. Therefore the main factor that influences LEV pharmacokinetics appears to be the type and amount of fiber in semisolidified ENs. Semi-solid ENs are often used in home care. Therefore, when the ENs used in patients receiving LEV in home care are changed from liquid to semi-solid types, the previously maintained plasma LEV concentration may decrease, thus carefully monitoring the plasma levels of LEV is necessary.

A limitation of this study is, that effect of semisolid ENs other than those used in this study on LEV pharmacokinetics was not examined. Various semisolid ENs are currently being marketed; however, studying the interaction of all ENs with LEV is a difficult task. Our findings indicated that the effect of ENs on LEV pharmacokinetics were influenced by viscosity and the type of semisolidifiers used. The interaction between various semisolidi-

Table 4: List of key components per 200 mL of liquid or 200 g of semisolid type of enteral nutrients

		Liquid type				Semisolid type		
		F2α®	**Liquid Racol® NF**	**Ensure liquid®**	**Renalen® LP**	**OS-1® Jelly**	**Semisolid Racol® NF**	**Terumeal® Soft**
Protein	Total amount (g)	10	8.8	7	3.2		8.8	9.0
	Composition (g)	Milk protein (8.0)	Milk casein (6.8)	Casein sodium (6.8)	Milk protein (UNK)		Milk casein (6.8)	Milk protein (UNK)
		Soy protein (2.0)	Soy protein isolate (3.3)	Soy protein isolate (1.0)			Soy protein isolate (3.3)	
Carbohydrate	Total amount (g)	30.2	31.2	27.4	59.2	5.0	31.2	45.8
	Composition (g)	Dextrin (UNK)	Maltodextrin (29.8)	Dextrin (19.6)	Dextrin (UNK)	Glucose (5.0)	Maltodextrin (29.8)	Dextrin (UNK)
		Sucrose (UNK)	Sucrose (2.6)	Sucrose (8)	Sucrose (UNK)		Sucrose (2.6)	Sucrose (UNK)
Lipid	Total amount (g)	4.4	4.5	7.0	9.0		4.5	9.0
	Composition (g)	Medium-chain triglyceride (UNK)			Medium-chain triglyceride (UNK)			Organic oil (UNK)
		Soybean oil (UNK)	Soybean oil (1.4)	Soy Lecithin (0.32)			Soybean oil (1.4)	
		Canola oil (UNK)			Canola oil (UNK)			
			Tricaprylin (1.5)				Tricaprylin (1.5)	
			Perilla oil (0.36)				Perilla oil (0.36)	
			Palm oil (0.66)		Palm oil (UNK)		Palm oil (0.66)	
				Corn oil (6.6)				
					Fish oil (UNK)			
Fiber (g)		Guar gum degradation (4.0)			Indigestible dextrin (3.2)	Unidentified (UNK)	Sodium alginate (0.5) Agar-agar (0.5)	Agar-agar (UNK)
Calorie (kcal)		200	200	200	320	20	200	300

UNK, unknown

fiers with high viscosity and LEV should be carefully examined in the future. In addition, if viscosity contributes to decreased absorption of LEV, fecal concentration of LEV may increase. We could not clarify the changes in fecal LEV excretion in this study, thus further investigation is required.

In summary, we clarified that co-administration of LEV with Terumeal® Soft decreased the gastrointestinal absorption of LEV. This phenomenon could be avoided by first administering LEV, waiting for 2 h, and then administering Terumeal® Soft. Moreover, semisolid Racol® NF altered the pharmacokinetics of LEV without decreasing its gastrointestinal absorption. Our findings suggested that carefully monitoring the plasma levels of LEV is necessary when co-administered with Terumeal® Soft, semisolid Racol® NF, or any other semisolid ENs, to prevent the inadvertent effects of the interaction between LEV and ENs.

4. Experimental

4.1. Chemicals

LEV was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Gabapentin, an internal standard, was purchased from Sigma-Aldrich (Tokyo, Japan). All ENs, including F2α® (Terumo Co. Ltd., Tokyo, Japan), liquid Racol® NF, semisolid Racol® NF (Otsuka Pharmaceutical Factory, Inc., Tokyo, Japan), Ensure Liquid® (Abbott Japan Co. Ltd., Tokyo, Japan), Renalen® LP (Meiji Seika Pharma Co., Ltd., Tokyo, Japan), OS-1 Jelly® (Otsuka Pharmaceutical Factory, Inc), and Terumeal® Soft (Terumo Co. Ltd.) were purchased from commercial sources. For animal anesthesia, sevoflurane was purchased from FUJIFILM Wako Pure Chemical Co., Ltd (Tokyo, Japan). Acetonitrile (Nacalai Tesque, Inc., Kyoto, Japan) was of chromatographic reagent grade, whereas all other chemicals were of analytical grade.

4.2. Animals

Fifty-five healthy male Sprague–Dawley rats (Japan SLC Inc., Shizuoka, Japan) weighing approximately 250 g each (7–8 week old) were used for the oral administration studies. Rats were housed in rooms under a controlled environment (temperature: 22±2 °C, humidity: 55±5 %, 12 h light/dark cycle, and diurnal time: 0800–2000 h) with food and water provided *ad libitum* for 1 week. All experimental procedures were conducted in accordance with the Osaka Ohtani University guidelines for the care and use of laboratory animals (approval no. 2004) and adhering to the ARRIVE guidelines.

4.3. Oral co-administration of LEV with ENs

Rats were cannulated via the right jugular vein under sevoflurane anesthesia 24 h before the drug absorption experiment. Rats were divided into 6 groups for liquid ENs and 5 groups for semisolid ENs. Liquid EN groups 1–4 were administered LEV in combination with one among the 4 types of liquid ENs (F2α®, liquid Racol® NF, Ensure Liquid®, or Renalen® LP), group 5 received only LEV with distilled water (control group), and group 6 received LEV and distilled water within 15 min of consuming laboratory Rodent Diet (Land O' Lakes, Inc, Minnesota, America), which is a complete solid food (solid food group). Semisolid EN groups 1–3 were administered LEV and one among the 3 types of semisolid ENs (OS-1® Jelly, semisolid Racol® NF, or Terumeal® Soft), group 4 received only LEV with distilled water (control group), and group 5 received Terumeal Soft® 2 h after an initial dose of LEV in distilled water (Terumeal® separate group). LEV (75 mg/kg) and 4 mL/kg of each EN were orally administered simultaneously, using a metal delivery device for intragastrointestinal administration. Plasma samples were collected through the jugular vein cannula at 0.25, 0.5, 1, 2, 3, 5, 7, and 9 h after LEV administration and replaced with an equal volume of saline. To maintain patency, a small volume of heparinized saline (MOCHIDA Pharmaceutical Co., Ltd., Tokyo, Japan) was used to fill the cannula after blood sample collection. The heparinized saline was removed immediately before blood sample collection. Rats were euthanized through deep anesthesia using sevoflurane after collection of the final blood sample. Plasma was obtained by centrifuging blood at 100 × *g* for 10 min at 4 °C. Samples were stored at –30 °C until further analysis. All efforts were made to minimize the suffering of rats.

4.4. Measurement of LEV concentration

LEV concentrations in the EN or plasma samples were determined using high-performance liquid chromatography (HPLC), as previously mentioned with some modifications (Pucci et al. 2004; Can and Arli 2010; Engelbrecht et al. 2017; Mendoza Aguilera et al. 2018). Briefly, 150 µL of an internal standard (gabapentin) and methanol was added to 50 µL of each sample. Samples were vortexed for 30 s and centrifuged at 11 000 × *g* for 20 min at 4 °C. Thereafter, 50 µL of the supernatant was transferred to a clean tube and 20 µL of the supernatant was injected into the HPLC system.

The HPLC system used was a Shimadzu VP-series consisting of an LC-40D pump, DGU-403 deaeration unit, CTO-40C column oven, SPD-M40 UV detector, and SIL-40C autoinjector controlled by an SCL-40 controller (Shimadzu Co., Kyoto, Japan). Separations were performed on a Cosmosil® $5C_{18}$-AR-II column (5 µm, 4.6 mm I.D. × 250 mm; Nacalai Tesque, Inc.), preceded by a run through the Cosmosil® $5C_{18}$-MS-II guard column (4.6 mm I.D. × 10 mm). The mobile phase consisted of a mixture of 50 mM KH_2PO_4 buffer, acetonitrile, and methanol (90:7:3, v/v). The pH of the mobile phase was adjusted to approximately 5.5 using sodium hydroxide (NaOH); the flow rate used was 1.0 mL/min with an injection volume of 20 µL. The column temperature was maintained at 35 °C and eluting peaks were monitored through measuring UV absorbance at 205 nm.

4.5. Acidity and viscosity measurements

The acidity and viscosity of each EN were determined using a pH meter (Seven Easy S20; Mettler Toledo Co., Ltd., Tokyo, Japan) and a conical plate rotational viscometer (TVE-22HT; Toki Sangyo Co., Ltd., Tokyo, Japan), respectively.

4.6. Statistical analyses

The pharmacokinetic parameters were calculated using Moment.xls ver. 1.0 (Tabata et al. 1999). Statistical analyses were performed using the JMP® Pro 14 software (SAS Institute Inc., Cary, NC, USA). All data are presented as the mean±standard deviation and analyzed using one-way analysis of variance with Tukey's multiple comparison test. The threshold for significance was set at $P < 0.05$.

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Conflicts of interests: The authors declare that they do not have any competing interests.

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