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Human disposition of L-theanine in tea or aqueous solution

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ABSTRACT

After consumption of tea, L-theanine enters systemic circulation and is assumed to enter the brain. Several human studies indicate that L-theanine influences brain functioning. Knowledge about the pharmacokinetics of L-theanine facilitates further study of this health effect. Volunteers received 25–100 mg of L-theanine as tea, as L-theanine-enriched tea, and as biosynthetic L-theanine in aqueous solutions. Plasma was analysed for L-theanine content after which data were fitted with a 1- compartment model. For all interventions, the lag time was approximately 10 min and half-lives of absorption and elimination were approximately 15 and 65 min respectively. After approximately 50 min, maximum plasma concentrations of between 1.0 and 4.4 mg/L were achieved. Maximum plasma concentration and area under the plasma-concentration–time curve were dose-proportional. This knowledge allows prediction of plasma concentrations for various dose regimens supporting further study of a health benefit of L-theanine.

1. Introduction

Green and black tea are brewed from dried leaves of the tea plant *Camellia sinensis*. Tea leaves contain numerous compounds, including caffeine and flavonoids, which dissolve when the leaves are infused with hot water. An interesting compound in tea leaves is the amino acid L-theanine (γ-glutamylethylamide) (Juneja et al., 1999). L-Theanine is almost solely found in tea plants (Juneja et al., 1999), only exists in the free (non-protein) form, and is the predominant free amino acid (1–2% of the dry weight of the leaves, 50% of the total free amino acids) in green and black tea (Juneja et al., 1999).

Various health effects have been associated with L-theanine, such as neuroprotective effects and improved attention (De Mejia et al., 2009). Both electroencephalographic [EEG] and behavioural studies indicate that L-theanine influences brain functioning. During rest, L-theanine increases α activity in the EEG (Palva and Palva, 2007), indicating greater relaxation as measured in different periods between 40 and 105 min following consumption in three studies (Kobayashi et al., 1998; Nobre et al., 2008; Song et al., 2003). During performance of a task that requires attention, L-theanine induced changes in α activity that indicated increased attentional processing measured 0–75 min (Gomez-Ramirez et al., 2007) and 30–100 min after consumption of L-theanine (Gomez-Ramirez et al., 2008).

Together with caffeine, L-theanine may have a synergistic positive effect on attention. This has been measured in an

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Abbreviations: $AUC_{70}^{0\rightarrow\infty}$, area under the plasma concentration–time curve from $t = 0$ to ∞ min corrected to a body weight of 70 kg; BW, body weight; C_p, plasma concentration; $C_{max,70}$, maximum plasma concentration corrected to a body weight of 70 kg; Cl/F_{abs}, clearance over the absolute bioavailability; D, dose; EEG, electroencephalography; k_{01}, absorption rate constant; k_{10}, elimination rate constant; $t_{½,abs}$, half-life time of absorption; $t_{½,el}$, half-life time of elimination; t_{lag}, lag time; t_{max}, time to maximum plasma concentration; V_{hyp}/F_{abs}, hypothetical volume of distribution over the absolute bioavailability

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EEG studies between 30 and 120 min (Kelly et al., 2008), and in a behavioural study after 60 min following L-theanine plus caffeine consumption (Owen et al., 2008). So, although there is some information on the time course of L-theanine effects from EEG and behavioural studies, knowing the pharmacokinetic profile of L-theanine would be a next step in better understanding the brain and behavioural effects of L-theanine.

Animal studies into the mechanism of action of absorption of L-theanine indicate that this compound is transported through the intestinal brush border via a Na^+-coupled co-transporter and hydrolysed to glutamic acid and ethylamine by the action of a phosphate-independent glutaminase in the kidney (Kitaoka et al., 1996; Tsuge et al., 2003; Unno et al., 1999). However, no data have been reported about the rate of clearance of L-theanine *in vivo*. Thus, data from animal studies indicate that in humans L-theanine is expected to be absorbed well, possibly followed by metabolism to glutamic acid and ethylamine in the kidney.

To our knowledge data on the pharmacokinetics of L-theanine have only been reported for animals. For example in mice L-theanine was detected in the brain 30 min after intraperitoneal injection of 7.7 mMol ^{14}C-L-theanine per kg body weight [BW] in the brain (Kimura & Murata, 1971). Terashima et al. (1999) presented studies in which rats ($n = 6$) were given 23 mMol L-theanine per kg BW intragastrically. L-Theanine plasma concentrations reached a maximum after 1 h (approximately 12 μmol/mL) and returned to baseline after about 24 h. L-Theanine concentration in the brain, however, reached a maximum of 1.5 μmol/mL after 5 h and was eliminated from brain tissue after 24 h. Data from animal studies suggest that for humans rapid absorption and elimination of L-theanine can be expected.

The source of L-theanine and the matrix in which it is administered might have an impact on its pharmacokinetics and absorption rate, respectively. Thus the aim of this work was to undertake a human study to investigate the pharmacokinetics of pure L-theanine from an aqueous solution, L-theanine from black tea, and L-theanine from L-theanine-enriched black tea. These beverages have negligible energy density allowing accurate estimation of the pharmacokinetics of L-theanine.

2. Materials and methods

2.1. Study design and study population

A randomised, non-blinded, crossover study was designed to study the pharmacokinetics of L-theanine in humans. Subjects were divided over five treatment sequences according to a partial Williams design (Williams, 1949).

Fifteen male volunteers, $\geqslant$18 and $\leqslant$28 years old, with a body mass index $\geqslant$18 and $\leqslant$32 kg/m^2, being in apparent good health and with results for clinical and haematological tests within normal reference were asked to participate in the study. Key exclusion criteria were blood donation in the month prior to and during the study, dieting, and high alcohol consumption ($\geqslant$28 units per week). Participants were asked to refrain from consumption of tea and tea-based products during a 48 h period before the start of the treatment. On the morning of intervention days, volunteers came to the facility while fasting.

The study was managed by the International Pharmaceutical Evaluation Center, Breda, The Netherlands. The study was carried out at Sta. Elena Hospital, Urb. Los Alamos, Torremolinos, Malaga, Spain. Ethical approval was obtained from the relevant medical ethical committee (Comité Andaluz de Ensayos Clínicos, Spain) and volunteer recruitment was carried out according to the guidelines as can be found in the Declaration of Helsinki 2000.

2.2. Study products and experimental procedure

In this study three sources of L-theanine were used. These were pure L-theanine (Suntheanine®, Taiyo Kagaku, Yokkaichi, Japan; 101 w/w% pure), commercial Lipton Yellow label (2 g tea leaves per tea bag, Lipton US, Englewood Cliffs, NJ, USA), and tea-derived L-theanine (L-theanine purified from green tea, Taiyo Kagaku, Yokkaichi, Japan, 68.7 w/w % pure).

For intervention I^a, I^b, and I^c 25, 50, and 100 mg pure L-theanine was dissolved in a cup of 314 g hot water, respectively. For intervention II, a cup of black tea (314 mL) was made from Lipton Yellow label in such a way that it (naturally) contained approximately 25 mg L-theanine. Intervention III, finally, was similar to the cup of black tea of intervention II in which 25 mg additional tea-derived L-theanine was dissolved. After allowing the drinks to cool down to approximately 60 °C, volunteers drank the beverage as fast as they could. A sample of 1 mL was collected for analysis and stored at −80 °C. On each intervention day, 1 of 5 drinks was consumed. The time at which the drink was administered was defined as $t = 0$ min.

Blood samples were collected from an indwelling canula in the antecubital vein in 6 mL sodium ethylenediaminetetraacetic acid tubes at $t = 0$, 15, 30, 45, 60, 90, 180, 240, 360, and 480 min. Subsequently the blood sample was centrifuged and two 1.5 mL plasma samples were prepared and stored at −80 °C.

2.3. Quantification of L-theanine in study products

First the sample was allowed to thaw. After homogenisation and filtration, L-theanine was separated from other amino acids and matrix compounds by HPLC on a Hypersil HyPURITY Elite C18, 5 μm, 150 mm × 4.6 cm (Phenomenex, Cheshire, UK) column. After injection ($t = 0$ min) solvent A (5 mM pentadecafluorooctanoic acid in water) and solvent B (5 mM pentadecafluorooctanoic acid in acetonitrile), were used in a gradient: 0–8 min 85% solvent A, linearly changing to 80% solvent A at $t = 10$ min, linearly changing to 10% solvent A at $t = 11$ min. Three minutes thereafter this was changed linearly to 85% solvent A at $t = 15$ min. After separation, L-theanine was derivatised using o-pthalaldehyde. This compound was detected using a RF2000 Fluorescence detector (Dionex, Camberley, UK) with excitation and emission set at 340 and 425 nm, respectively. The linear range of this method was 1–160 pg/mL. The precision and reproducibility were 5% and 2 % relative SD, respectively.

2.4. Quantification of L-theanine in plasma

A method was developed to quantify L-theanine in plasma, based on L-theanine-d_5 as internal standard (custom synthesis by Sigma–Aldrich/Isotec, St. Louis, MO, USA).

Briefly, to each 200 μL plasma sample 1000 μL acetonitrile were added; liquids were mixed and the precipitated material was removed by centrifugation. The supernatant was collected and evaporated under nitrogen at room temperature. The dried extracts were re-dissolved in 200 μL water. A Waters Alliance HPLC (Waters, Shanghai, China) equipped with a 3.9 × 150 mm, 4 μm particle size Nova-Pack C18 column (Waters, Shanghai, China) was used. Compounds were eluted isocratically using a phase of water containing 0.00003 w/v % ammonia, 0.5 v/v % formic acid, and 2 v/v % acetonitrile at a flow of 0.4 mL/min. The eluent was connected to the electron spray ionisation source of a Micromass Quattro mass spectrometer (Waters, Shanghai, China). Ionisation was carried out in positive mode with 3.2 kV capillary voltage and a cone voltage of 20 V. Source temperature was 100 °C and desolvation temperature was 350 °C. Nitrogen was used as cone gas and as desolvation gas at a flow of 50 and 350 L/h, respectively.

For all multiple reaction monitoring settings the dwell time was 0.50 s, the collision energy 10.0 eV, and a cone voltage of 20.0 eV. L-Theanine-d_5 was identified by the ratio of the m/z 180.10 > 50.80 and 180.10 > 163.00 signals. For L-theanine this was m/z 175.00 > 45.80 and 175.00 > 158.00. Both compounds were quantified by using the first transition.

In short this method allowed quantification of L-theanine in plasma between 5 and 500 μg/mL. The coefficient of variation of the within run reproducibility and the between run reproducibility were <5% and <10%, respectively.

2.5. Pharmacokinetic analysis

The L-theanine plasma concentrations were weighted uniformly. The plasma concentrations observed in a single experiment were modelled using a 1-compartment model with first-order input and output and lag time (Gabrielsson & Weiner, 2000):

$$C_p = \frac{F_{abs} \cdot D \cdot k_{01}}{V_{hyp} \cdot (k_{01} - k_{10})} \cdot \left[e^{(-k_{10} \cdot (t - t_{lag}))} - e^{(-k_{01} \cdot (t - t_{lag}))} \right] \quad (1)$$

where C_p = plasma concentration [mg/L], D = dose [mg], k_{01} = absorption rate constant [per min], V_{hyp}/F_{abs} = hypothetical distribution volume over absolute bioavailability [L], k_{10} = elimination rate constant [per min], t = time [min], and t_{lag} = lag time [min].

The following primary parameters were estimated V_{hyp}/F_{abs}, k_{10}, k_{01}, and t_{lag} using Gauss–Newton minimization with Levenberg and Hartley modification (Hartley, 1961); next, the following secondary parameters were calculated (Gabrielsson & Weiner, 2000): absorption half-life time [$t_{½,a}$; min], elimination half-life time, [$t_{½,e}$; min], maximum plasma concentration corrected to a BW of 70 kg [$C_{max,70}$; mg/L], time of maximum plasma concentration [t_{max}; min], clearance over absolute bioavailability [Cl/F_{abs}; L/min], and the area under the plasma concentration–time curve from t = 0 to ∞ min corrected to a BW of 70 kg [$AUC_{70}{}^{0\rightarrow\infty}$; mg/L·min].

WinNonlin® software (version 6.1, Pharsight, Mountain View, CA, USA) was used for pharmacokinetic analyses.

2.6. Statistics

Values for pharmacokinetic parameters were excluded if they differed >3*SD from the average of an intervention. In general, values are presented as average ± SD.

Differences between the interventions were analysed by a simple linear model using ANOVA in which the subjects acts as replicates. Differences were defined being statistically significant at $p < 0.05$. The dose–response relationships were based on data from all matrices and sources of L-theanine.

SAS/Stat software, Version 9.2 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA) was used for statistical calculations.

3. Results

3.1. Study population and L-theanine measurements

In total 15 healthy males were included being 18–28 years old and weighing 79.4 ± 15.6 kg (ranging from 58.0 to 112.8 kg). All volunteers completed the study and were compliant with the study restrictions as L-theanine concentrations in baseline samples were below the limit of quantification.

L-Theanine doses as administered during the five interventions are presented in Table 1. Pearson's correlation coefficients of standard curves of the method for determination of L-theanine in plasma were >0.99. This allowed accurate construction of 75 L-theanine plasma concentration–time curves.

A typical individual L-theanine plasma concentration–time curve after consumption (t = 0 min) of a cup of black tea containing 22 mg L-theanine is presented in Fig 1.

3.2. Pharmacokinetic analysis

In total 73 out of 75 plasma concentration–time curves could be modelled. At the population level there was no trend in the scatter of observed – predicted concentrations (data not shown). One curve from intervention I^a and I^b could not be modelled because the model did not converge and 1 outlier was detected (I^c). As a summary the pharmacokinetic profile of L-theanine in healthy male humans is presented in Table 1.

For L-theanine doses ranging approximately from 20 to 100 mg, the relationship with BW-corrected maximum L-theanine plasma concentration was $C_{max,70}$ [mg/L] = 0.045 × L-theanine dose [mg] − 0.073 (R^2 = 0.89, n = 72; see Fig. 2a). For L-theanine doses ranging approximately from 20 to 100 mg, the relationship and BW-corrected area under the plasma concentration–time curve was $AUC_{70}{}^{0\infty}$ [min·mg/L] = 5.783 × L-theanine dose [mg] + 5.293 (R^2 = 0.87, n = 72; Fig. 2b).

3.3. Statistical analysis

One value for V_{hyp}/F_{abs} and 1 for Cl/F_{abs} (related to the same subject) were identified as outliers and therefore excluded. The p-value for lag time was 0.63, for $t_{½,a}$ 0.02, for t_{max} 0.02, for $t_{½,e}$ 0.07, for V_{hyp}/F_{abs} 0.04, and for Cl/F_{abs} 0.76.

Table 1 – The pharmacokinetic profile of L-theanine.[a].

Intervention[c]	Parameter[b]							
	Dose [mg]	$t_{½,a}$ [min]	t_{max} [min]	$C_{max,70}$ [mg/L]	$t_{½,e}$ [min]	$AUC_{70}^{0\rightarrow\infty}$ [min·g/L]	Cl/F_{abs} [L/min]	V_{hyp}/F_{abs} [L]
I[a]	24.9 ± 1.7	14 ± 10	46 ± 14	1.06 ± 0.22	67 ± 18	0.15 ± 0.03	0.20 ± 0.04	18 ± 3
I[b]	49.9 ± 1.6	10 ± 4	43 ± 9	2.22 ± 0.45	64 ± 9	0.28 ± 0.05	0.21 ± 0.03	19 ± 3
I[c]	98.6 ± 1.8	9 ± 4	41 ± 9	4.43 ± 0.70	66 ± 8	0.58 ± 0.10	0.20 ± 0.05	19 ± 3
II	23.1 ± 1.2	21 ± 10	55 ± 13	0.97 ± 0.18	55 ± 14	0.14 ± 0.02	0.20 ± 0.05	15 ± 3
III	45.2 ± 2.2	14 ± 7	51 ± 11	1.94 ± 0.32	70 ± 17	0.28 ± 0.05	0.19 ± 0.04	18 ± 5

[a] Healthy young males received three types of L-theanine-containing beverages: pure L-theanine dissolved in an aqueous solution, black tea, L-theanine-enriched black tea. A 1-compartment model with first-order input and output and lag time was used to assess pharmacokinetic parameters. Lag time across all interventions was about 8–10 min. Values are means ± SD, $n = 15$ (Intervention I[a], I[b], and I[c]: $n = 14$).

[b] $t_{½,a}$, absorption half-life time; t_{max}, time of maximum plasma concentration; $C_{max,70}$, maximum plasma concentration corrected to a body weight of 70 kg; $t_{½,e}$, elimination half-life time; $AUC_{70}^{0\rightarrow\infty}$, area under the plasma concentration–time curve from $t = 0$ to ∞ min corrected to a body weight of 70 kg; Cl/F_{abs}, clearance over absolute bioavailability; V_{hyp}/F_{abs}, hypothetical distribution volume over absolute bioavailability.

[c] Hot water (314 g) to which either 25 mg (I[a]), or 50 mg (I[b]), or 100 mg pure L-theanine was added (I[c]), a cup of black tea of 314 g, naturally containing about 25 mg L-theanine, to which either 0 (II) or 25 mg (III) tea-derived L-theanine was added. The time tea was administered was defined as $t = 0$ min.

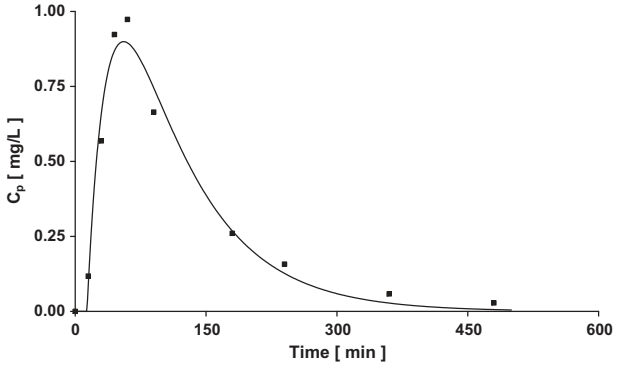

Fig. 1 – Typical individual plasma L-theanine concentration–time curve after consumption ($t = 0$ min) of a cup of black tea containing 22 mg L-theanine (Intervention II). Symbols are observed L-theanine plasma concentration [Cp]. A 1-compartment model with first-order input and output including lag time was used to predict L-theanine plasma concentrations which are presented by a line.

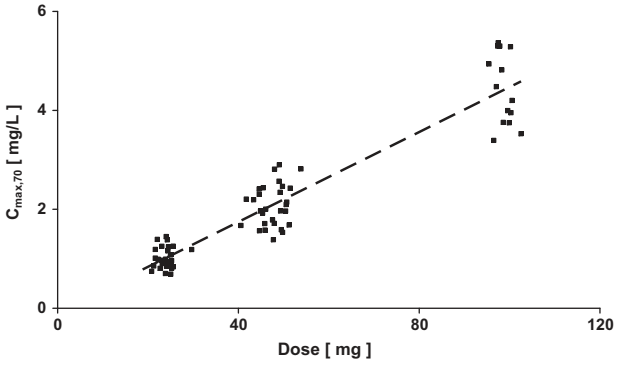

Fig. 2a – Relationship between body weight-corrected maximum L-theanine plasma concentration and L-theanine dose using data from all interventions. Symbols represent single observations ($n = 72$). The dashed line represents linear regression of the data: $C_{max,70}$ [mg/L] = 0.045 × L-theanine dose [mg] − 0.073; R2 = 0.89.

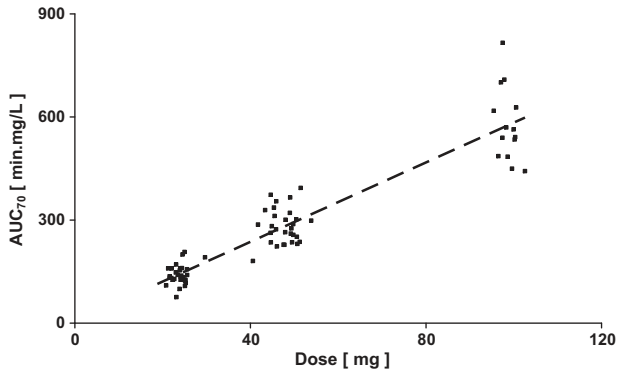

Fig. 2b – Relationship between body weight-corrected area under the plasma concentration–time curve and L-theanine dose using data from all interventions. Symbols represent observations ($n = 72$). The dashed line represents linear regression of the data: AUC_{70} [min·mg/L] = 5.78 × L-theanine dose [mg] + 5.293, $R^2 = 0.87$.

4. Discussion

To the best of our knowledge this is the first pharmacokinetic study of L-theanine in humans from various sources of L-theanine, as administered from either an aqueous solution or from black tea. In all cases, L-theanine was absorbed quickly and resulted in maximum plasma concentrations of up to approximately 5 mg/L L-theanine. The pharmacokinetics could be described with a simple model and linear first-order kinetics. Dose response parameters AUC_{70} and $C_{max,70}$ were linear with L-theanine doses equivalent to that of 1–4 cups of tea. The L-theanine doses in this study were of the same order of magnitude as those applied in the study of Kobayashi about the effects of L-theanine on the release of α-brain waves (Kobayashi et al., 1998). This knowledge about pharmacokinetic behaviour of L-theanine, allows the prediction of L-theanine concentration in the systemic circulation in a tea consumption-relevant dose range. The ability to

predict L-theanine concentration may facilitate research about putative health effects of L-theanine in the brain.

Dose-independent pharmacokinetic parameters are fairly consistent across the various doses, L-theanine source and matrices. Only 1 out of 75 experiments was identified as outlier. The pharmacokinetic fate of L-theanine in humans starts with liberation of L-theanine from the matrix. For aqueous solutions like water or tea almost immediate release can be expected as illustrated by lag times of 5–10 min and this is indeed reflected in the nearly identical t_{max} and $C_{max,70}$ values for the tea or water administration events. Dose-dependent parameters were consistent as well after correction for BW.

The design of the study did not allow assessment of the absolute bioavailability of L-theanine. On the other hand, we determined values of V_{hyp}/F_{abs} which were found to be 15–20 L (Table 1). These rather low values, close to total plasma volume (or body water) values indicate that for this matrix, L-theanine uptake is significant. The absolute bioavailability of L-theanine, an amino acid, is however expected to be close to 100%. Experimentally, this value can only be assessed by administering known L-theanine doses both intravenously and orally. Once L-theanine enters systemic circulation it will be cleared predominantly in the kidney, being an amino acid (Kitaoka et al., 1996; Tsuge et al., 2003; Unno et al., 1999). It can be calculated that if four cups of tea are consumed as quickly as possible, clearance of L-theanine (Cl/F_{abs} about 0.20 ± 0.04 L/min) from systemic circulation will take place within about 8 h.

There could be a weak dose dependency in the half-life times of both absorption and elimination of L-theanine. The rapid uptake of L-theanine is likely to be due to the low energy density of the food matrix resulting in a fast transit of the stomach content to the duodenum for absorption. In rats, L-theanine has been shown to be absorbed from the small intestine into the blood stream by common Na^+-coupled co-transporters in the intestinal brush-border membrane (Kitaoka et al., 1996). Results obtained in rats (Unno et al., 1999) also indicate that L-theanine is absorbed rapidly: the first plasma samples taken 15 min after oral administration contained high concentrations of L-theanine, and maximal plasma concentrations were reported after 45 min. In rats, maximal plasma concentrations also occurred approximately 30 and 45 min after an oral dose of 200 mg per kg BW (Unno et al., 1999) or 500 mg per kg BW (Desai et al., 2005), respectively.

Relationships between doses and $C_{max,70}$ and $AUC_{70}{}^{0\rightarrow\infty}$ were consistently linear across the dose range (Figs. 2a and 2b). At doses equivalent to 0.31, 0.63 and 1.25 mg per kg BW the average maximum plasma concentrations in the volunteers were 0.94, 2.08 and 3.93 mg/L L-theanine, respectively. This is consistent with the data in rats, where plasma concentration also increased linearly with dose: after a dose of 100, 200 and 400 mg per kg BW plasma concentrations of approximately 200, 560 and 810 mg/L , respectively, were reported by Unno et al. (1999). Thus, dose linearity was also reported in rats even though the doses given to the animals were 300 times higher than those given to our human subjects, suggesting that even a dose of 400 mg L-theanine per kg BW does not approach maximal capacity of the gastrointestinal tract (of a rat) to absorb L-theanine.

The obtained set of pharmacokinetic parameters from this study allow accurate prediction of L-theanine concentrations in plasma over a relevant dose range. These predictions allow assessment of L-theanine concentrations in the systemic circulation as a function of time and dose. The impact of the composition of other food matrices like sugar, milk and especially concomitantly ingested meals on the pharmacokinetic behaviour of L-theanine is unknown and could be studied in future research.

5. Conclusions

L-Theanine obtained from various sources (pure, biosynthetic, and tea-derived) is quickly absorbed and eliminated from the systemic circulation. The pharmacokinetic behaviour of L-theanine, independent of source, is very similar for simple matrices like water and tea. The pharmacokinetic model can be used to correlate plasma kinetics within the average tea-consumption dose range with potential benefits of L-theanine. The model will facilitate the design of studies related to L-theanine effects.

Conflict of interest

Joseph, Chen, Mulder and Van der Pijl are full time employees of Unilever which markets products containing L-theanine.

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