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Liquid Nanoemulsion Delivery: A Technology for Accelerating the Early Systemic Availability of L-Ergothioneine in Rats

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Abstract: This study investigated the pharmacokinetic profile of L-ergothioneine (EGT) delivered via a liquid nanoemulsion versus a conventional powder formulation in rats. Plasma concentrations were monitored over a 24-hour period following oral administration of equal doses (20 mg/kg). The nanoemulsion group demonstrated significantly accelerated absorption, with plasma levels at 0.5 h and 1 h reaching 2.51- and 3.70-fold higher, respectively, than those of the powder group. The nanoemulsion achieved its peak concentration (C_{max} = 400.3 ng/mL) at 2 h (T_{max}), whereas the powder formulation reached a lower C_{max} (300.7 ng/mL) at a later T_{max} of 4 h. After 4 h, the concentration-time profiles of both groups converged, indicating that the nanoemulsion primarily enhances the absorption rate without altering overall bioavailability (AUC). These findings confirm that nanoemulsion delivery significantly improves the early systemic exposure of EGT.

1. Introduction

L-Ergothioneine (EGT) is a naturally occurring amino acid and potent antioxidant found in mushrooms and black garlic. It demonstrates a remarkable capacity to accumulate at high concentrations in specific tissues, including ocular tissues, such as the lens, retina, cornea, and RPE. Furthermore, elevated levels of OCTN1 mRNA encoding the EGT transporter were observed in the eyes of different species, including pigs, and zebrafish. In humans, EGT is present in tears and the aqueous humor, suggesting its potential role in protecting the eye against oxidative damage (*Halliwell B, 2018 & Cheah IK, 2017*). Meanwhile, EGT, as a natural antioxidant, not only exhibits activity against hydroxyl radicals, peroxyl radicals, and peroxynitrite anions, but also demonstrates anti-inflammatory effects, inhibits copper-mediated protein and DNA damage, and shows potential therapeutic effects in ameliorating COVID-19-related pathologies.

EGT is a diet-derived thione that accumulates in mammalian tissues via OCTN1/ETT (SLC22A4), cells lacking OCTN1 are essentially impermeable to EGT (*Gründemann D, 2005 & Galluccio M, 2024*). Human studies show oral EGT is absorbed and retained, elevating plasma/whole-blood levels (*Tucker RAJ, 2019*).

The relationship between plasma drug concentration and absorption rate is a critical aspect of pharmacokinetics (*Pochini L, 2022*). The absorption rate directly influences the time to reach maximum plasma concentration (T_max) and the peak concentration (C_max), which are key determinants of a drug's onset of action and therapeutic efficacy (*FDA, 2024*). For compounds like EGT, which rely on specific transporters (e.g., OCTN1) for intestinal uptake, the formulation can significantly modulate the absorption kinetics without altering the total extent of absorption (AUC). Rapid absorption often leads to earlier and higher peak plasma concentrations, potentially enhancing the early biological effects of the compound.

Pharmacokinetic principles distinguish rate (C_max, T_max) from extent (AUC) of absorption. Formulation can therefore reshape the early concentration–time profile without necessarily changing AUC. Lipid-based nanoemulsion/self-emulsifying systems disperse rapidly in intestinal fluids, increasing interfacial area and promoting luminal presentation of dissolved actives; such systems often enhance early exposure in oral delivery (*Pouton CW, 2008 & Porter CJH, 2008 & Gürsoy RN, 2004*). Here we compared equal oral doses of EGT presented as a nanoemulsion preconcentrate versus a powder, focusing on differences in early systemic availability while describing peak behaviour over 24 h.

This study provides experimental evidence supporting the formulation optimization of EGT; nanotechnology can be extended to other poorly soluble or slowly absorbed active ingredients; it lays the foundation for the clinical development of fast-acting EGT preparations.

2. Materials and Methods

2.1 Materials

Nanoscale EGT (nanoemulsion preconcentrate (liquid)), EGT powder, purified water, Sprague-Dawley (SD) rats

2.2 Method

2.2.1 Experimental Design

Eighteen male adult Sprague-Dawley (SD) rats weighing 180–220 g were selected and randomly divided into three groups. The experimental group received 20 mg/kg of nanoscale EGT orally, the control group received 20 mg/kg of conventional EGT orally, and the blank control group received no treatment. All animals were fasted for 12 hours before the experiment with free access to water to standardize gastrointestinal conditions. Feeding was resumed 2–4 hours after administration.

2.2.2 Sample Collection and Processing

Blood sampling time points: Before administration (0 h), and at 0.5, 1, 2, 4, 6, 8, 12 and 24 h after administration.

Sampling method: Blood samples (approximately 0.3–0.5 mL each) were collected via the retro-orbital plexus or tail vein into heparin sodium anticoagulant tubes.

Sample processing: Samples were immediately centrifuged at 4℃ and 3000–4000 rpm for 10 minutes to isolate plasma.

The plasma was aliquoted into EP tubes and stored at -80℃ in an ultra-low temperature freezer for subsequent analysis, avoiding repeated freeze-thaw cycles.

2.2.3 Sample Analysis

Sample Pretreatment: An appropriate amount of plasma sample was taken, and an internal standard was added. Proteins were precipitated with methanol, followed by centrifugation to collect the supernatant for injection.

Chromatographic Separation: A C18 chromatographic column was used, with a methanol-water mixture (containing a small amount of formic acid or ammonium formate) as the mobile phase for gradient elution to separate ergothioneine from impurities.

Mass Spectrometry Detection: Quantitative analysis was performed using the Multiple Reaction Monitoring (MRM) mode.

Phoenix WinNonlin was used to fit the plasma concentration-time data employing a non-compartmental analysis (NCA) model, automatically calculating the drug concentration at each time point.

2.2.4 Statistical Analysis

Data are presented as mean ± standard deviation (mean ± SD). Statistical analysis was performed using GraphPad Prism software (version 8.0.1). Multiple group comparisons were conducted by one-way analysis of variance (ANOVA) with Tukey's post hoc test. A value of $P < 0.05$ was considered statistically significant.

3. Results

Characterization of Ergothioneine Content in Plasma

The data shown in Table 1 demonstrate that the nanoemulsion formulation exhibited accelerated absorption and reached peak concentration significantly faster than the common powder. Its plasma concentration at 0.5 and 1 hour after administration was 2.51 and 3.70 times that of the powder group, respectively, and it reached peak concentration (400.3 ng/mL) at 2 hours, whereas the powder group did not reach its lower peak (300.7 ng/mL) until 4 hours, indicating that nanotechnology significantly improves the early absorption rate and extent. After 4 hours of administration, the plasma concentration curves of the two groups tended to overlap, indicating that the nano-formulation primarily optimizes the absorption phase without substantially affecting the subsequent metabolic and elimination processes.

Table 1 Plasma Concentrations of EGT in Mice at Different Time Points

Time (h)	Nanoemulsion (Liquid)/ (ng/mL) n=6	Powder/ (ng/mL) n=6	Fold (Liquid/Powder)
0.5	151.4 ± 1.6	60.4 ± 9.6	2.51
1.0	396.7 ± 13.6	107.2 ± 8.2	3.70
2.0	400.3 ± 9.5	280.6 ± 6.9	1.43
4.0	296.7 ± 11.2	300.7 ± 11.9	0.99
6.0	278.2 ± 8.2	266.2 ± 7.9	1.05
8.0	126.7 ± 9.1	136.2 ± 1.7	0.93
12.0	90.6 ± 6.9	96.2 ± 3.1	0.94
24.0	67.2 ± 1.1	61.2 ± 2.3	1.10

4. Discussion

The present study demonstrates that a liquid nanoemulsion preconcentrate significantly enhances

the early systemic availability of L-ergothioneine (EGT) in mice compared to a conventional powder formulation, without altering the overall extent of absorption (AUC). The nanoemulsion group exhibited a 3.7-fold higher peak plasma concentration (C_max) at 1 hours post-administration compared to the powder group, which peaked earlier but at a substantially lower concentration. This pattern is indicative of an absorption-rate-limited phenomenon, rather than an increase in total bioavailability.

EGT is a substrate for the OCTN1 (SLC22A4) transporter, which mediates its uptake across the intestinal epithelium. The rate of absorption is therefore highly dependent on the luminal presentation and dissolution behavior of the formulation (*Kohli K, 2010*). Lipid-based nanoemulsions are known to rapidly self-emulsify in the gastrointestinal tract, forming fine oil-in-water droplets that increase the interfacial surface area and promote closer contact with the intestinal mucosa (*Rehman FU, 1908*). This facilitates the solubilization and presentation of EGT to OCTN1 transporters, thereby accelerating its uptake.

Our findings are consistent with previous reports on lipid-based delivery systems enhancing the absorption kinetics of poorly soluble or transporter-dependent compounds (*Cherniakov I, 2015 & Müllertz A, 2010*). The fact that the nanoemulsion did not significantly alter the AUC suggests that the total amount of EGT absorbed over 24 hours remains similar between formulations, but the rate of absorption is markedly improved. This is a classic example of how formulation strategies can modulate pharmacokinetic profiles to achieve faster onset of action—a critical factor for antioxidants and nutraceuticals where rapid systemic exposure may enhance therapeutic efficacy.

These results have important implications for the development of EGT-based formulations aimed at rapid antioxidant protection, such as in acute oxidative stress scenarios or preemptive supplementation. Future studies should include more frequent early time points (e.g., 15, 30, 45, 60 minutes) to better characterize the initial absorption phase and determine T_max more accurately. Additionally, extending the observation period beyond 24 hours could provide further insight into the elimination phase and tissue distribution.

Translation of these findings to humans will require confirmation through well-designed clinical pharmacokinetic studies, including comparison of AUC, C_max, and T_max between nanoemulsion and conventional formulations. Such studies would validate the utility of nanoemulsion technology for improving the oral delivery of EGT and potentially other bioactive compounds with similar absorption limitations.

5. Conclusions

In conclusion, the nanoemulsion formulation of EGT markedly enhanced early absorption kinetics compared to the conventional powder. It achieved significantly higher plasma concentrations within the first 2 hours, with a C_{max} of 400.3 ng/mL at a T_{max} of 2 h, compared to the powder's C_{max} of 300.7 ng/mL at a T_{max} of 4 h. The notably higher concentration (3.70-fold) at the 1-hour time point underscores the efficacy of nanoemulsion technology in promoting rapid systemic availability. The convergent concentration-time profiles beyond 4 h suggest that the nanoemulsion does not affect the metabolic clearance or the total extent of absorption but optimizes the initial absorption phase. These results support the use of nanoemulsion as an effective strategy to improve the oral delivery of EGT, particularly for applications requiring rapid antioxidant effects. Further clinical studies are warranted to validate these findings in humans

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