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Physiological Mechanism-Based Analysis of Dose-Dependent Gastrointestinal Absorption of L-Carnitine in Rats

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ABSTRACT: We evaluated the dose-dependent (saturable) gastrointestinal absorption of L-carnitine, a lipid-lowering agent, in rats by a physiological mechanism-based approach to clarify its absorption characteristics and to examine the *in vitro* (*in situ*)–*in vivo* correlation in intestinal transport. The intestinal absorption rate constant (k_a), which was estimated by the analysis of gastrointestinal disposition, decreased markedly from 0.1061 to 0.0042 min^{-1} when the dose was increased from 0.05 μmol rat^{-1} (low dose) to 100 μmol rat^{-1} (high dose). The dose-dependence in k_a was attributable to the saturability of intestinal transport that, in the perfused intestine, was similar to the saturability in k_a. At the high dose, the apparent absorption rate constant (k'_a) of 0.0021 min^{-1}, which was estimated by the analysis of plasma concentrations after oral administration, was an order of magnitude smaller than the gastric emptying rate constant (k_g) of 0.059 min^{-1} and comparable with the k_a of 0.0042 min^{-1}, suggesting that the gastrointestinal absorption of L-carnitine is absorption-limited in the intestine. At the low dose, where intestinal L-carnitine absorption was far more efficient, the k'_a of 0.0172 min^{-1} was smaller than the k_a of 0.1061 min^{-1} and closer to the k_g of 0.072 min^{-1}, suggesting that apparent absorption was retarded by gastric emptying which is less efficient than intestinal absorption. This shift in the rate-determining process with an increase in dose explains the less marked dose dependence in k'_a compared with k_a. The bioavailability decreased from 100 to 42% with an increase in dose. This could be accounted for quantitatively by a reduction in the fraction absorbed ($F_{a,oral}$) due to a reduction in k_a, assuming first-order absorption during the transit time of T_{si} through the small intestine ($F_{a,oral} = 1 - \exp(-k_a \cdot T_{si})$). Thus, using L-carnitine as a model, this study has successfully demonstrated that the saturability in gastrointestinal absorption can be correlated with the intestinal transport in a quantitative and mechanism-based manner. This should be of help not only for developing more efficient oral L-carnitine delivery strategies, taking advantage of *in vitro* (*in situ*) information about the intestinal transport mechanism, but also for establishing a more generally applicable *in vitro* (*in situ*)–*in vivo* correlation in gastrointestinal absorption. © 1998 John Wiley & Sons, Ltd.

Key words: intestinal absorption; carrier-mediated transport; L-carnitine; gastrointestinal disposition; rat

Introduction

L-Carnitine is a γ-amino acid which serves as an essential cofactor for the transfer of long-chain fatty acids across the inner mitochondrial membrane in which β-oxidation occurs [1] and, therefore, it has been used to treat organic acidemias [2]. Interest has been increasing in its potential application to treat a variety of symptoms related to the metabolism of fatty acids [1,3]. However, at current pharmacological doses, its oral absorption is incomplete, making oral therapy less efficient. It is also known that the oral absorption of L-carnitine is dose-dependent [4], potentially leading to dose-dependent variability in bioavailability. Although the involvement of carrier-mediated transport in L-carnitine absorption has been demonstrated *in vitro* [5–7] and *in situ* [8], and suggested to be a cause of dose-dependent absorption *in vivo*, no quantitative *in vitro* (*in situ*)–*in vivo* correlation has yet been established.

We, therefore, evaluated the dose-dependent gastrointestinal absorption of L-carnitine *in vivo* using a physiological mechanism-based approach, and our own method for the analysis of gastrointestinal drug disposition after oral administration [9]. Characterizing *in vivo* absorption in detail and clarifying its quantitative relationship with *in vitro* (and *in situ*) intestinal transport should be helpful in developing more efficient oral delivery strategies, particularly in terms of *in vivo* absorption predicted from information gathered *in vitro* (and *in situ*).

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Materials and Methods

Chemicals

L-[N-methyl-^{14}C]Carnitine hydrochloride (2.0 GBq mmol^{-1}) and [^{3}H]PEG 4000 (0.069 GBq g^{-1}) were purchased from Dupont-NEN Co. (Boston, MA). Soluene-350, a tissue solubilizer, and Scintisol EX-H, a scintillation cocktail, were purchased from Packard Instrument Co., Inc. (Meriden, CT) and Dojindo Lab. (Kumamoto, Japan), respectively. Unlabeled L-carnitine hydrochloride (Sigma Chemical Co., St. Louis, MO) and urethane (Tokyo Kasei Kogyo Co., Tokyo, Japan) were obtained commercially. All other reagents were of analytical grade and obtained commercially.

Animals

Male Wistar rats, weighing about 300 g, were used after fasting overnight, unless otherwise indicated.

Analysis of Gastrointestinal Disposition

Solutions for administration were prepared in saline (0.9% NaCl solution), and contained 0.05 mM [^{14}C]L-carnitine for the low dose solution and 100 mM L-carnitine with a tracer amount of [^{14}C]L-carnitine for the high dose solution. [^{3}H]PEG 4000 was also added as a nonabsorbable marker at a tracer concentration (0.19 mg mL^{-1}).

The rats were given oral doses, 1 mL rat^{-1}, of 0.05 mM (low dose: 0.05 μmol rat^{-1}) or 100 mM (high dose: 100 μmol rat^{-1}) L-carnitine with 0.19 mg mL^{-1} (0.19 mg rat^{-1}) of [^{3}H]PEG 4000, using a gastric tube. The rats were then placed in a metabolic cage at the ambient temperature of 23°C, allowed unrestricted movement and sacrificed at 10, 20, 40, or 60 min after dosing by puncturing the heart under ether anesthesia. Sampling of the gastrointestinal contents and tissues of the stomach, duodenum and three equal length segments of the small intestine (jejunum, midgut and ileum) was carried out as described in our previous report [9]. After adding the appropriate amount of saline, the gastrointestinal contents and tissues were homogenized, and a portion of each homogenate was solubilized to determine the radioactivity [9], using Soluene-350 (1 mL) as a tissue solubilizer and Scintisol EX-H (5 mL) as a scintillation cocktail.

Part of each contents sample from the animals receiving the high dose was analysed by TLC to determine the intact L-carnitine as follows. Fifty microlitres of sample was added to 250 μL 0.3 M KOH in methanol for deproteinization, followed by heating at 56°C for 1 h [10]. Then, deproteinized supernatant was obtained by centrifugation at 4°C and 15000 × *g* for 10 min in an MRX-150 centrifuge (Tomy Seiko Co., Tokyo, Japan). The supernatant was passed through a disposable filter (DISMIC-13CP 0.45 μm, Advantec Co., Tokyo, Japan) before subjecting an aliquot (50 μL) to TLC: adsorbent, silica-gel 60 F_{254}; plate size, 5 × 20 cm; layer thickness, 0.25 mm; solvent system, methanol/28% ammonia solution (1/1) [11]; development time, 1 h. The radioactivity was determined with an imaging analyser, BAS 2000 (Fuji Photo Film Co., Ltd., Tokyo, Japan).

The fraction of L-carnitine recovered from each segment of the gastrointestinal tract was estimated as the sum of that in the contents sample and in the fluid adhering to tissue [9]. The volume of adhering fluid was estimated by dividing the amount of PEG 4000, a nonabsorbable marker, associated with the tissue sample by its concentration in the contents sample.

The fraction of L-carnitine recovered was normalized by the total fraction of the PEG 4000 dose recovered from the gastrointestinal tract to correct for minor fluctuations in sampling. The corrected values, represented as the fraction remaining (FR), were subjected to the following model analysis.

Assuming that solute transfer is defined by gastric emptying and subsequent intestinal absorption is governed by first-order rate constants k_g and k_a, respectively, the fraction of the dose remaining in the stomach (FR_s) and small intestine (FR_{si}) can be described as a function of time (t) as follows [9]:

$$FR_s = e^{-k_g \cdot t} \quad (1)$$

$$FR_{si} = (e^{-k_a \cdot t} - e^{-k_g \cdot t})/(1 - k_a/k_g) \quad (2)$$

Equations (1) and (2) were simultaneously fitted to FR_s and FR_{si} data for L-carnitine to estimate k_a and k_g, using a nonlinear regression program, PCNONLIN (Scientific Consulting, Inc., Cary, NC), and weighted according to the reciprocal of the variance. As discussed in our previous report [9], although these model equations are mathematically the same as those for a linear compartment model consisting of the stomach and small intestine compartments, it is unnecessary to retain the compartment model assumption that there is even drug distribution in each compartment. However, it is assumed that the apparent intestinal membrane permeability clearance for unit length (Cl_{app}) and the average intestinal lumen volume for unit length (V_{av}) have constant values along the small intestine and k_a represents the ratio of Cl_{app} to V_{av}.

Pharmacokinetic Analysis of Plasma Concentration Data

The rats had a cannula inserted into the right jugular vein under light ether anesthesia. After regaining consciousness and allowing a recovery period of 1 h, each rat was given orally (through a gastric tube) or intravenously (through the cannula) 1 mL rat^{-1} of 0.05 mM (low dose: 0.05 μmol rat^{-1}) or 100

mM (high dose: 100 μmol rat^{-1}) L-carnitine, placed in a metabolic cage at an ambient temperature of 23°C and allowed to move freely; 0.25 mL blood was taken periodically through the cannula, placed in a centrifuge tube containing 5 units of heparin and centrifuged for 3 min in a Microfuge E (Beckman Instruments, Inc., Palo Alto, CA) to obtain plasma. The plasma (50 μL) was placed in a counting vial, to which was added 5 mL Scintisol EX-H to determine the radioactivity by liquid scintillation counting. Part of each plasma sample was analysed by TLC to determine intact L-carnitine as described for the gastrointestinal contents in the high dose animals.

Plasma concentration (C) versus time (t) profiles of L-carnitine were analysed by a two-compartment model with first-order absorption, where the plasma concentrations after intravenous and oral administration are described by Equations (3) and (4), respectively [12]:

$$C = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \quad (3)$$

$$C = L \cdot e^{-\alpha \cdot t} + M \cdot e^{-\beta \cdot t} - N \cdot e^{-k'_a \cdot t} \quad (4)$$

$$L = \frac{A \cdot F \cdot k'_a}{(k'_a - \alpha)} \quad (5)$$

$$M = \frac{B \cdot F \cdot k'_a}{(k'_a - \beta)} \quad (6)$$

$$N = L + M \quad (7)$$

where A, B, α and β are constants, and k'_a and F are the apparent absorption rate constant and bioavailability, respectively. The values of A, B, α and β were estimated by fitting Equation (3) to the concentration versus time profiles after intravenous administration, using PCNONLIN. With the values of A, B, α and β fixed, the values of k'_a and F were estimated by fitting Equation (4) to the concentration versus time profiles after oral administration. The half-life of the β phase ($t_{1/2\beta}$), distribution volume of the central compartment (V_{dl}), steady-state distribution volume (V_{dss}) and total body clearance (Cl_{tot}) were calculated from A, B, α and β [12].

In Situ Intestinal Perfusion Experiments

Perfusion solutions were prepared in phosphate buffer (20.1 mM Na_2HPO_4, 47.0 mM KH_2PO_4, 101.0 mM NaCl; pH 6.4), and contained L-carnitine (0.01 or 10 mM) with a tracer concentration of [^{14}C]L-carnitine. A tracer concentration of [^{3}H]PEG 4000 was also added as a nonabsorbable marker. Intestinal single-pass perfusion was carried out in rats not fasted prior to the experiments and anesthetized with urethane (1.25 g kg^{-1}, i.p.), as previously described [13–15], using a 10 cm midgut segment and a perfusion rate of 0.15 mL min^{-1}. The outflow solution was collected for 20 min at 5 min intervals, starting 25 min after the start of perfusion, by which time steady-state had been achieved.

Scintillation cocktail (Scintisol EX-H, 5 mL) was added to the 50 μL aliquots of inflow and outflow solutions to determine the radioactivity by liquid scintillation counting.

The fraction of L-carnitine absorbed (F_a) was estimated as the fraction disappearing from the intestinal lumen, corrected for a minor volume change based on the change in PEG 4000 concentration.

The preepithelial diffusional resistance, which generates a concentration gradient dropping towards the intestinal surface in the water phase adjacent to the intestinal surface, may affect the permeability estimates and this was taken into account by using a model which incorporated an unstirred water layer (UWL) [13–15]. The intestinal membrane permeability clearance (Cl_m) was estimated from the concentration at the intestinal surface, using the following equations:

$$\frac{1}{Cl_{app}} = \frac{1}{Cl_m} + \frac{1}{Cl_{aq}} \quad (8)$$

$$Cl_{app} = -\frac{Q}{L} \cdot \ln(1 - F_a) \quad (9)$$

$$Cl_{aq} = 2\pi R \cdot \frac{D}{\delta} \quad (10)$$

where Cl_{app} and Cl_{aq} are the apparent membrane permeability clearance and the permeability clearance of the UWL, respectively; Q is the flow rate; L and R are the length and radius of the perfused segment, respectively; D and δ are the diffusion coefficient and the effective thickness of the UWL, respectively. The D value of L-carnitine was calculated to be 7.44×10^{-6} cm^2 s^{-1} from that of D-glucose (7.04×10^{-6} cm^2 s^{-1}) [16], assuming that D is inversely proportional to the square root of the molecular weight [17]. Using this D value, a δ of 952 μm [15] and an R of 0.234 cm [13], the Cl_{aq} of L-carnitine was calculated using Equation (10) and, estimating Cl_{app} from Equation (9), Cl_m was calculated using Equation (8).

Gastric Absorption

The rats were anesthetized with urethane (1.25 g kg^{-1}, i.p.), and the low dose (0.05 μmol rat^{-1}) of L-carnitine was administered into the closed stomach which was washed internally and ligated at the cardia and pylorus. The amount of L-carnitine (radioactivity) remaining in the gastric contents was determined 60 min after administration as described previously by liquid scintillation counting [18].

Biliary Excretion

In an unfasted state, the rats were anesthetized with urethane (1.25 g kg^{-1}, i.p.). The common bile duct was cannulated with PE-10 tubing, and bile was collected for 60 min after introduction (1.0 mL) of

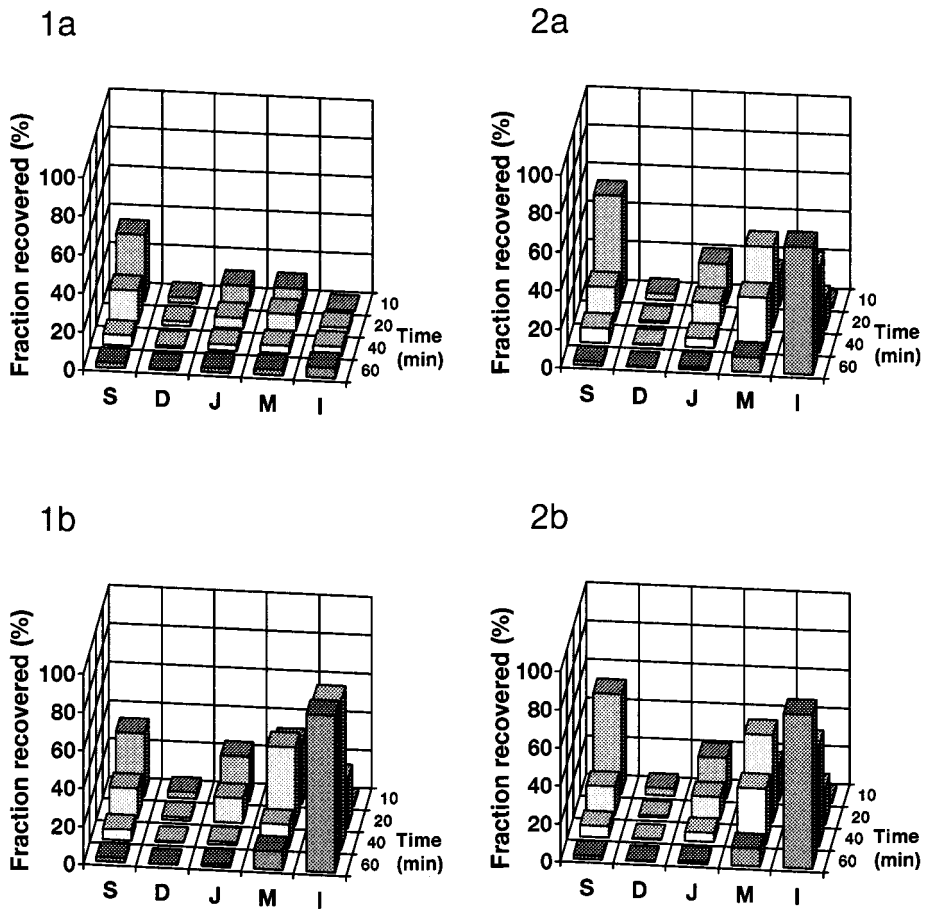

Figure 1. Gastrointestinal distribution of L-carnitine and coadministered PEG 4000 after oral administration in rats. Panels (1a) (L-carnitine) and (1b) (PEG 4000) are for the low dose (0.05 μmol rat^{-1}), and panels (2a) (L-carnitine) and (2b) (PEG 4000) are for the high dose (100 μmol rat^{-1}). Results are represented as the average value ($n = 4$). S, stomach; D, duodenum; J, jejunum; M, midgut; I, ileum

0.01 mM [^{14}C]L-carnitine solution, which was prepared in phosphate buffer (pH 6.4) containing added [^{3}H]PEG 4000 as a nonabsorbable marker, into a 10 cm intestinal (midgut) loop. The amount of L-carnitine (radioactivity) excreted in bile was determined as described previously by liquid scintillation counting [19]. At the end of the experiments, L-carnitine remaining in the intestinal lumen was also determined to evaluate L-carnitine absorption (disappearance) from the loop.

Statistical Analysis

Levels of statistical significance were assessed using Student's *t*-test.

Results and Discussion

Gastrointestinal Distribution Profiles

As shown in Figure 1, the distribution profile of L-carnitine was similar in shape to that of PEG 4000 at every sampling time for both low and high doses, suggesting that the intestinal transit of L-carnitine can be represented by that of PEG 4000. The fractional recovery of L-carnitine was comparable with that of PEG 4000 in the stomach at every sampling point, suggesting insignificant gastric absorption. In the small intestine, the recovery of L-carnitine was lower than that of PEG 4000, but increased with dose, suggesting the involvement of saturable intestinal transport. The total recovery of PEG 4000 from the stomach and small intestine was almost 100% (95.9 ± 2.3 and 93.5 ± 2.3%, respectively, for the low and high doses expressed as a mean $\pm$ S.E. for $n = 4$) even 60 min after administration, suggesting that the distribution of the administered compounds, including L-carnitine as well as PEG 4000, was restricted within the region of the gastrointestinal tract and any transit from the small intestine to the large intestine was negligible.

Although it has been reported that L-carnitine may be degraded to some extent by intestinal micro flora [10], no degradation was detected even in the ileum 60 min after administration of the high dose (data not shown). For the low dose, the bioavailability was about unity, as described later, indicating complete absorption without significant loss due to bacterial degradation. Thus, L-carnitine does not seem to be degradable in the small intestine.

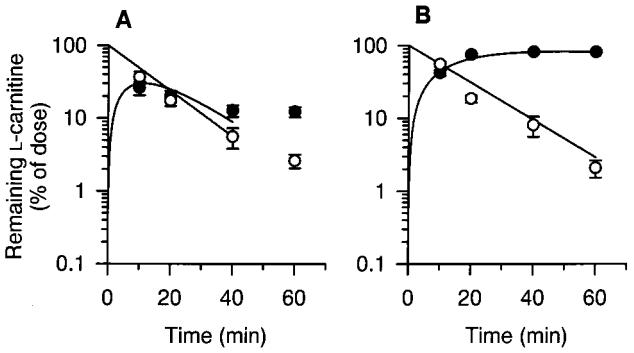

Figure 2. Remaining L-carnitine versus time profiles for the stomach (○) and small intestine (●) after oral administration of L-carnitine in rats. Results are represented as the mean ± S.E. ($n = 4$). The solid lines represent the computer-fitted profiles. A, low dose (0.05 μmol rat^{-1}); B, high dose (100 μmol rat^{-1})

Insignificant gastric absorption and biliary excretion of L-carnitine were confirmed in rats anesthetized with urethane as follows. The gastric absorption from the closed stomach was only 1.5 ± 1.1% (mean ± S.E.; $n = 3$) over a period of 60 min. Also, the biliary excretion was only 0.005 ± 0.003% (mean ± S.E.; $n = 3$) of the dose (or about 0.006% of the absorbed amount) 60 min after administration to the closed midgut loop. This negligible biliary excretion is consistent with the finding that there was no apparent delay in the shift in the distribution of L-carnitine, compared with that of PEG 4000 (Figure 1).

All these results are consistent with the assumptions in the model analysis incorporating only gastric emptying and intestinal absorption (Equations (1) and (2)).

Kinetic Analysis of Gastrointestinal Disposition

For the model analysis, the remaining fractions of L-carnitine from all intestinal segments were summed for each time to obtain the total fraction of L-carnitine remaining in the small intestine, and normalized by the total recovery of PEG 4000 from the gastrointestinal tract to correct for minor fluctuations in sampling. The remaining fraction versus time profiles of L-carnitine for stomach and small intestine were successfully described by the presented model (Equations (1) and (2)) up to 40 and 60 min, respectively, for the low and high doses (Figure 2). For the low dose, the data at 60 min could not be predicted by the model, but absorption was almost (about 90%) complete by 40 min. Therefore, they were excluded from the analysis. After administration of the 0.05 mM solution for the low dose, luminal L-carnitine concentrations should be lower than 0.05 mM, and may be comparable with physiological concentrations in plasma (0.015–0.025 mM) [20]. However, because carrier-mediated (active) transport was suggested to be predominant for L-carnitine absorption at the low dose as discussed later, L-carnitine could be absorbed against a concentration gradient. After 40 min, luminal L-carnitine concentrations were presumably too low to maintain, even by active transport, net absorption that could be approximated by unidirectional absorption flux.

As summarized in Table 1, while the k_g was not affected by dose, the k_a of L-carnitine was markedly reduced with an increase in dose, in agreement with earlier suggestions of the involvement of saturable (carrier-mediated) intestinal transport *in vitro* [5–7] and *in situ* [8]. Thus, the present study successfully demonstrates a dose-dependence in intestinal L-carnitine absorption *in vivo*, and k_a can be evaluated as a parameter that is not hybridized by gastric emptying. It should also be noted that, at the high dose, the intestinal absorption rate constant (k_a) was about ten times smaller than the gastric emptying rate constant (k_g), suggesting that the gastrointestinal absorption of L-carnitine is absorption-limited in the intestine. That was not the case at the low dose where k_a was comparable with k_g.

In Situ–In Vivo Correlation in Intestinal Transport

It has been suggested that in the rat small intestine L-carnitine is absorbed predominantly by carrier-mediated transport, with a Michaelis constant of about 0.2–1.3 mM, at concentrations below 0.1 mM and predominantly by passive transport at concentrations above 5 mM [7,8]. In the present *in vivo* study, the L-carnitine concentrations were 0.05 and 100 mM, respectively, in the low and high dose solutions, and lower in the intestinal lumen after oral administration because of dilution by luminal fluid and removal by absorption. Therefore, for the

Table 1. Dose-dependence in gastrointestinal disposition parameters in rats

Dose (μmol rat^{-1})	k_g (min^{-1})	k_a (min^{-1})	$Cl_{app,vivo}$ (μL min^{-1} cm^{-1})	Cl_m (μL min^{-1} cm^{-1})
0.05 (low)	0.072 ± 0.007	0.1061 ± 0.0126	2.55	1.90 ± 0.25
100 (high)	0.059 ± 0.003	0.0042 ± 0.0009	0.10	0.18 ± 0.04

The values of k_g (gastric emptying rate constant) and k_a (intestinal absorption rate constant) are represented as computer-fitted parameters with their corresponding S.E. $Cl_{app,vivo}$, apparent membrane permeability clearance *in vivo*; Cl_m, membrane permeability clearance *in situ* determined at 0.01 and 10 mM, respectively, for the low and high doses and represented as the mean ± S.E. ($n = 3$).

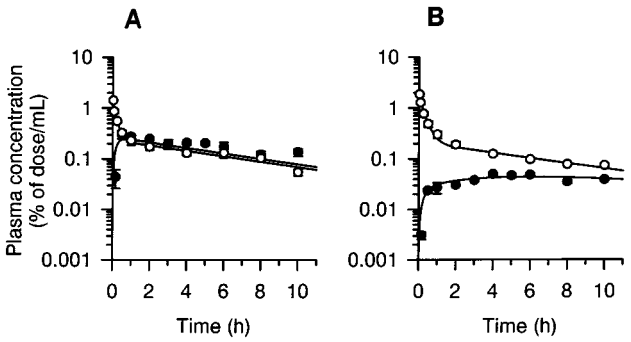

Figure 3. Plasma concentrations of L-carnitine after intravenous (○) and oral (●) administration in rats. Results are represented as the mean ± S.E. ($n = 3$). The solid lines represent the computer-fitted profiles. A, low dose (0.05 μmol rat^{-1}); B, high dose (100 μmol rat^{-1})

low dose (0.05 μmol rat^{-1}), it can be assumed that the luminal concentrations were much lower than the Michaelis constant and that carrier-mediated transport was predominant and followed linear kinetics. For the high dose (100 μmol rat^{-1}), the lowest concentration for the major sites of distribution (absorption) of about 12.5 mM was observed at 40 min in the jejunum (Figure 1), where about 5% of the dose was distributed in a jejunal volume of about 0.4 mL [9]. Hence, it can be assumed that passive transport was predominant.

To examine the *in situ–in vivo* correlation in intestinal L-carnitine transport, the membrane permeability clearance (Cl_m) was evaluated in the perfused rat intestine at a low concentration (0.01 mM), where carrier-mediated transport would be predominant, and at a high concentration (10 mM), where passive transport would be predominant. The values of Cl_m are listed in Table 1 with the values of $Cl_{app,vivo}$ as the product of k_a and the average luminal volume (V_{av}, 24 μL cm^{-1} from our previous report [9]). The Cl_m value at the low concentration was an order of magnitude larger than that at the high concentration, consistent with earlier reports [7,8]. For both low and high concentrations, the Cl_m value was comparable with the $Cl_{app,vivo}$ value. These results strongly suggest that the saturability in intestinal absorption *in vivo* is solely due to intestinal membrane transport.

Pharmacokinetic Analysis of Plasma Concentrations

For both doses, the plasma concentration versus time profiles after intravenous and oral administration were successfully described by a two-compartment model and a two-compartment model with first-order absorption, respectively (Figure 3), and the kinetic parameters are listed in Table 2. The delay in distribution (reduction in α) with an increase in dose may be due to saturability of carrier-mediated uptake in the liver and in cardiac and skeletal muscle [1,20]. The reduction in V_{dl} suggests saturation in distribution to an instantaneously accessible space or tissue, e.g. binding to endothelial cells, although this still remains to be documented.

At the high dose, the k'_a of 0.0021 min^{-1} was an order of magnitude smaller than the k_g of 0.059 min^{-1} and comparable with the k_a of 0.0042 min^{-1}, supporting the suggestion of intestinal absorption-limited absorption by analysis of gastrointestinal disposition. This was not the case at the low dose, where intestinal L-carnitine absorption was more efficient; the k'_a of 0.0172 min^{-1} was smaller than the k_a of 0.1061 min^{-1} and closer to the k_g of 0.072 min^{-1}, suggesting that apparent absorption was retarded by gastric emptying that was less efficient than intestinal absorption. Thus, although k'_a was also highly dose-dependent, it was less marked than for k_a, and can be explained by a shift in the rate-determining process with an increase in dose.

The bioavailability (F) fell markedly from 100 to 42% with an increase in dose. These F values were comparable with the orally absorbed fractions ($F_{a,oral}$) of 100 and 28%, respectively, for the low and high doses predicted from the combination of the k_a values of 0.1061 and 0.0042 min^{-1}, respectively, and small intestinal transit time (T_{si}) of 78 min from our previous study [21], assuming that $F_{a,oral}$ is, at each dose, defined by linear absorption in the small intestine ($F_{a,oral} = 1 - e^{-k_a \cdot T_{si}}$) [21,22]. Thus, it is suggested that the dose-dependence of F can be quantitatively accounted for by that of $F_{a,oral}$ due to the saturable intestinal transport. This result also suggests that the first-pass metabolism of L-carnitine is negligible. This is in agreement with earlier reports that L-carnitine was mainly eliminated by renal excretion [4,10], and our findings that L-carnitine was not degraded in the small intestine (discussed earlier) and no metabolite was detected in plasma during the earlier phase up to 2 h for both low and high doses.

Table 2. Dose-dependence in the pharmacokinetic parameters of L-carnitine in rats

Parameters	Dose	
	0.05 μmol rat^{-1}	100 μmol rat^{-1}
A (% of dose mL^{-1})	1.12 ± 0.08	1.44 ± 0.05*
B (% of dose mL^{-1})	0.23 ± 0.04	0.21 ± 0.02
α (h^{-1})	5.01 ± 0.29	3.20 ± 0.48*
β (h^{-1})	0.12 ± 0.01	0.12 ± 0.01
$t_{1/2\beta}$ (h)	5.75 ± 0.65	6.03 ± 0.79
V_{d1} (mL kg^{-1})	285 ± 14	234 ± 10*
V_{dss} (mL kg^{-1})	1375 ± 197	1185 ± 197
Cl_{tot} (mL h^{-1} kg^{-1})	185 ± 16	169 ± 1
k'_a (min^{-1})	0.0172 ± 0.0023	0.0021 ± 0.0003**
F	1.000 ± 0.00001	0.422 ± 0.046**

Data are represented as the mean ± S.E. ($n = 3$). Levels of statistical significance compared with the value for the low dose (0.05 μmol rat^{-1}): * $p < 0.05$; ** $p < 0.01$.

Although L-carnitine is known to be acylated in various tissues including the liver, and present, to some extent, in acylated form in plasma, it seems reasonable that L-carnitine does not undergo significant first-pass metabolism, including acylation, for the following reasons: (i) L-carnitine is least likely to be acylated in the intestinal lumen because acylation is known to be mediated by acyltransferases, that exist in various tissues but not in the intestinal lumen [23]; (ii) L-carnitine reportedly does not undergo significant acylation in the intestinal mucosa [7]; (iii) we did not observe significant accumulation of radioactivity in intestinal tissue after oral administration of [^{14}C]L-carnitine in the present study; (iv) it is reported that radioactivity does not accumulate extensively in the liver after enteral administration of [^{3}H]L-carnitine [20]; (v) it is reported that orally administered L-carnitine at a pharmacological dose initially causes a rise in the concentration of unacylated L-carnitine in plasma without affecting the acylated forms that exist physiologically [24]. These facts suggest that L-carnitine is transferred from the intestinal lumen to the systemic circulation without being significantly acylated or temporarily accumulating in the intestinal mucosa or liver during its first pass. However, in the later phase after 2 h, metabolites were detected in plasma in moderate amounts (below 30%) for the high dose and in lesser amounts for the low dose. These metabolites may be acylated forms of L-carnitine that were released from some tissues following a delay after uptake of L-carnitine and acylation. For the high dose, they may be in part bacterial degradation products absorbed in the large intestine [10]. However, undegraded L-carnitine does not seem to be absorbed in the large intestine as the *F* values could be predicted by assuming absorption only in the small intestine.

Conclusions

The dose-dependent (saturable) gastrointestinal absorption of L-carnitine, with regard to both the extent and rate of absorption, can be successfully characterized in rats by the physiological mechanism-based approach, and is quantitatively and solely attributable to saturability of carrier-mediated intestinal transport. Considering that oral L-carnitine absorption has also been reported to be highly saturable in humans [4,6], similar to our results in the rat, the *in vitro* (*in situ*)–*in vivo* correlation determined in the present study should help guide research into the development of more efficient oral L-carnitine delivery strategies, taking advantage of *in vitro* (and *in situ*) information about transport mechanisms. It should also help in establishing *in vitro* (*in situ*)–*in vivo* absorption correlations that are generally applicable to a variety of drugs.

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