Fractional Absorption of L-Carnitine after Oral Administration in Rats: Evaluation of Absorption Site and Dose Dependency

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We evaluated the fractional absorption of L-carnitine, a γ-amino acid essential cofactor for the transfer of long-chain fatty acids, in rats *in vivo* after oral administration to determine its absorption behavior. At both low (0.05 μmol/rat) and high (100 μmol/rat) doses, L-carnitine was recovered only from the region of the cecum and below at 10 h after administration. During a major shift in distribution from cecum at 10 h to feces at 24 h, there was no significant change in the total recovery at each dose, suggesting that L-carnitine absorption is negligible in the cecum and the large intestine (colon and rectum). However, the recovery of L-carnitine was incomplete and the fraction recovered was larger at the high dose than at the low dose. The fractions absorbed were estimated to be 96.7 and 33.0% for the low and high doses, respectively, as these were the fractions that disappeared from the gastrointestinal tract. These values were comparable with 100 and 42%, respectively, of bioavailability values by the pharmacokinetic analysis of plasma concentration data in our preceding study [Matsuda *et al.*, *Biopharmaceutics & Drug Disposition*, in press]. These results suggest that L-carnitine is significantly absorbed only in the small intestine, without undergoing first-pass degradation, and in a dose-dependent manner presumably due to the involvement of saturable transport by L-carnitine carriers. Consistent with the suggestions *in vivo*, L-carnitine absorption in the closed intestinal loop *in situ* was concentration-dependent in the small intestine but not in the large intestine, and the apparent membrane permeability in the large intestine was smaller by an order of magnitude than that of passive transport in the small intestine. These findings support our preceding kinetic modeling strategy assuming the small intestine to be the sole absorption site, and should be of help in guiding studies on development of more efficient oral L-carnitine delivery strategies.

Key words fractional absorption; L-carnitine; dose dependency; rat; small intestine; large intestine

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]; it therefore has been used in the treatment of organic acidemias.[2] However, although it has also been the focus of increasing interest with regard to its potential applications for various symptoms related to the metabolism of fatty acids,[1,3] the oral absorption of L-carnitine is incomplete at current pharmacological doses, making oral therapy less efficient.[4—6] It is also known that the oral absorption of L-carnitine is dose-dependent,[4,5] potentially leading to dose-dependent variability in bioavailability. Therefore, development of more efficient oral L-carnitine delivery strategies requires characterization of the dose-dependent absorption and identification of the absorption mechanism.

Several studies *in vitro*[7—9] and *in situ*[10] have suggested that the carrier-mediated intestinal transport is a likely source of dose dependency in oral L-carnitine absorption *in vivo*, though little has been attempted to correlate with *in vivo* absorption quantitatively. In an attempt to verify those suggestions in terms of quantitative *in vitro* (*in situ*)–*in vivo* correlation, we previously evaluated dose dependency in bioavailability in rats and suggested that it was quantitatively accountable by dose (concentration)-dependent transport in the small intestine.[11] This finding, in turn, implicitly indicates that the large intestine would not be involved in L-carnitine absorption and that the bioavailability would be equivalent to the fraction absorbed (namely, first-pass degradation is negligible), and these issues have been left unverified.

Therefore, to further substantiate the preceding finding, we analyzed in this study the disposition of L-carnitine in the lower gastrointestinal tract (cecum and below) after oral administration to rats to determine the absorption site, examining if L-carnitine absorption is negligible in the large intestine. We also estimated the fraction absorbed to be that which disappeared from the gastrointestinal tract to evaluate dose dependency in fractional absorption and learn whether it is comparable with that in bioavailability. The transport of L-carnitine was also evaluated in the closed intestinal loop *in situ*, comparing between the small and the large intestines.

MATERIALS AND METHODS

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

Gastrointestinal Disposition *in Vivo* Dosing solutions for low and high doses were prepared in saline (0.9% NaCl solution), and contained 0.05 mM [^{14}C]L-carnitine and 100 mM L-carnitine with [^{14}C]L-carnitine at a trace level (0.025 mM), respectively. [^{3}H]PEG 4000 was also added as a nonabsorbable marker at a trace level (0.19 mg/ml). The rats, weighing about 300 g and fasted overnight, were orally given 1 ml of the 0.05 mM or 100 mM L-carnitine solution (0.05 and 100 μmol/rat, respectively, for the low and high doses), using a gastric tube. The animals were then left free in a metabolic

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cage at the ambient temperature of 23 °C, and sacrificed at 10 or 24 h after dosing by puncturing the heart under ether anesthesia to sample the gastrointestinal contents and tissues of stomach, duodenum, three equal lengths of small intestinal segments (jejunum, midgut and ileum), cecum and large intestine (colon and rectum), as described.[12] After adding an appropriate amount of saline, the gastrointestinal contents and tissues were homogenized, and a portion of each homogenized sample was solubilized for the determination of radioactivity, using Soluene-350 (1 ml) as a tissue solubilizer and Scintisol EX-H (5 ml) as a scintillation cocktail.

Feces were weighed in a counting vial, and homogenized in saline with a spatula to make a 20% homogenate. Roughly 100 mg of the homogenized sample was placed in the same type of vial and was solubilized to determine radioactivity in the same manner as tissue samples.

The fraction of L-carnitine (radioactivity) dose recovered (*FR*) from each segment of the gastrointestinal tract was estimated as the sum of that in the contents sample and that in the fluid adhering to the tissue. The volume of adherent fluid was estimated by dividing the amount of tissue-associated PEG 4000 (radioactivity) by the concentration in the contents sample.

Absorption in Intestinal Loop *in Situ* Rats weighing about 300 g and without fasting, were anesthetized with urethane (1.25 g/kg, i.p.). The absorption of L-carnitine was evaluated in the 5-cm intestinal loop of small intestine (midgut) or large intestine (colon) of the rats, as described.[13] L-Carnitine solutions (0.005, 0.01, 0.05, 10 and 100 mM) were prepared in phosphate buffer (pH 6.4), and trace amounts of [^{14}C]L-carnitine and [^{3}H]PEG 4000 (nonabsorbable marker) were added. For 100 mM solution, NaCl concentration was reduced to maintain isotonicity. Each experiment was started by the injection of a L-carnitine solution (0.5 ml) into the loop, and lasted for 60 min. For the lower concentrations of 0.005, 0.01 and 0.05 mM in the small intestine, experiments were terminated at 30 min because L-carnitine absorption was more efficient, presumably due to the significant contribution of carrier-mediated transport as described later. At the end of each experiment, the remaining L-carnitine and PEG 4000 in the loop were measured by radioactivity determination.

The fraction absorbed (F_a) was estimated as that which had disappeared from the intestinal lumen, correcting for minor volume changes based on changes in PEG 4000 concentration. Assuming a first-order absorption of L-carnitine, the absorption rate constant (k_a) was estimated as follows:

$$k_a = -\frac{\ln(1-F_a)}{t} \quad (1)$$

where t represents the absorption (experimental) period. The apparent membrane permeability clearance (CL_{app}) was estimated as the product of k_a and the luminal volume (100 μl/cm as administered volume).

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

RESULTS AND DISCUSSION

Evaluation of Absorption from Gastrointestinal Disposition *in Vivo* A low or high dose of L-carnitine was orally administered with a trace amount of PEG 4000 (nonabsorbable marker) to rats and, to focus on disposition in the region of the cecum and below, the recoveries of L-carnitine and PEG 4000 from the gastrointestinal tract and feces were evaluated at 10 and 24 h after administration. The high dose (100 μmol/rat or 333 μmol/kg) was within the range of pharmacological dose (161—689 μmol/kg) in humans,[14] and the low dose (0.05 μmol/rat) was to evaluate L-carnitine absorption at the maximal efficiency that can be expected for physiological L-carnitine intake. At the low dose, L-carnitine absorption was suggested to be at the maximal efficiency with predominant carrier-mediated transport, as discussed later in more detail.

Table 1. Total Recovery of L-Carnitine and Coadministered PEG 4000 from the Cecum, Large Intestine and Feces after Oral Administration to Rats

L-Carnitine dose (μmol/rat)	Time (h)	Recovery (% of dose)	
		L-Carnitine[a]	PEG 4000
0.05	10	4.5±0.6 (5.4±0.6)	83.5±2.7
	24	3.9±0.5 (4.2±0.5)	92.7±0.8
100	10	66.5±2.9 (73.9±3.3)	90.1±1.2
	24	64.5±8.3 (64.3±7.1)	99.5±3.0

Data are represented as the mean±S.E. (n=4). A trace amount of PEG 4000 was administered with L-carnitine. *a*) Values in parentheses are those normalized by the total recovery of PEG 4000 in each set of experiments.

At both 10 and 24 h after administration in the low and high L-carnitine dose experiments, the recoveries of L-carnitine and PEG 4000 from the stomach and the small intestine were negligible at undetectable levels. In the region of cecum and below, a major shift in distribution from the cecum to feces was observed between 10 and 24 h for both L-carnitine and PEG 4000 in the experiments of low and high L-carnitine doses (Fig. 1). During the major shift in distribution, however, there was no significant reduction in the total fraction of dose recovered for either L-carnitine or PEG 4000 at any L-carnitine dose (Table 1), suggesting that the absorption of both substances was negligible in the cecum and the large intestine. Therefore, the lower fractional recovery of L-carnitine than of PEG 4000 (nonabsorbable marker) at each dose is attributable to the absorption of L-carnitine in the small intestine. The larger fractional recovery of L-carnitine at the high dose, namely, reduced fractional absorption at this dose, is presumably due to the involvement of saturable transport by L-carnitine carriers.[7—10]

The total recovery of PEG 4000 was less than 100%, but only 8.5% lower on average from the 4 sets of experiments in Table 1. Although we cannot exclude the possibility that PEG 4000 may have been slightly absorbed in the small intestine, we assumed that the apparent loss could be attributable to minor differences between the nominal and the actual dose and loss in sample treatments. Practically, the total recoveries of L-carnitine at 10 and 24 h were, after normalization with that of PEG 4000, not significantly different at each L-carnitine dose, still implying that L-carnitine absorption is negligible in the cecum and the large intestine.

There was no significant difference between the fractions of total recovery distributed between L-carnitine and PEG 4000 at any sampling site at any L-carnitine dose and time (Fig. 1). This result suggests that L-carnitine was transferred from the cecum to feces, and presumably also in the upper

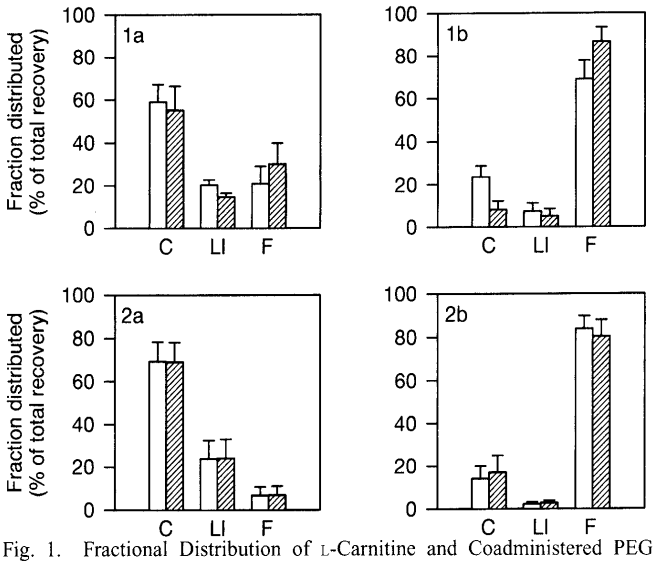

Fig. 1. Fractional Distribution of L-Carnitine and Coadministered PEG 4000 after Oral Administration to Rats

Panels 1a (10 h) and 1b (24 h) are for the low L-carnitine dose (0.05 μmol/rat), and panels 2a (10 h) and 2b (24 h) are for the high L-carnitine dose (100 μmol/rat). A trace amount of PEG 4000 was administered with L-carnitine. Data are represented as the mean±S.E. (n=4). The distributions of L-carnitine and PEG 4000 are shown by the open and hatched columns, respectively. Keys: C, cecum; LI, large intestine (colon and rectum); F, feces.

gastrointestinal tract, at the same fractional transfer rate (or transit velocity) as PEG 4000. If so, any fraction of PEG 4000 dose excreted in feces is equivalent to the fraction of L-carnitine dose originally emptied from the stomach with that fraction of PEG 4000. Therefore, the ratio of the fraction of L-carnitine dose excreted to that of PEG 4000 (namely, the fraction of L-carnitine dose originally emptied from the stomach) represents the fraction remaining (or unabsorbed) of this cofactor. As long as the transit time of small intestine (the site of absorption) is constant, as suggested in our preceding study on D-xylose,[15] the L-carnitine/PEG 4000 ratio of fraction of dose excreted would be constant for any fraction of dose and would represent the fraction remaining of the total dose. Therefore, the orally absorbed fraction of L-carnitine dose can be estimated by subtracting the L-carnitine/PEG 4000 ratio (or percentage) in feces from unity (or 100%), using any fraction of a dose.

In the present study, the L-carnitine/PEG 4000 ratio of fraction recovered in feces was 3.3±0.4 and 67.0±5.6% (mean±S.E., n=4), respectively, for the low and high doses as determined at 24 h, when significant amounts of each substance were recovered from every rat. Subtracting these values from 100%, the orally absorbed fractions of L-carnitine dose ($F_{a,oral}$) were calculated to be 96.7 and 33.0%, respectively. These $F_{a,oral}$ values were comparable with 100 and 42%, respectively, of bioavailability values for the low and high doses by the pharmacokinetic analysis of plasma concentration data in our preceding study.[11] It should be noted that the L-carnitine/PEG 4000 ratio in feces was comparable with the total recoveries of L-carnitine at each dose (Table 1), in agreement with the suggestion that L-carnitine was distributed only in the region of the cecum and below and that L-carnitine absorption was negligible in that region.

Thus, regardless of dose, L-carnitine absorption was found to be negligible in the cecum and the large intestine and the fraction absorbed (which disappeared from the gastrointestinal tract) was comparable with the bioavailability. These results suggest that L-carnitine is significantly absorbed only in the small intestine, without undergoing significant first-pass degradation. We evaluated the disposition of total radioactivity as that of L-carnitine, without any further analysis of intact L-carnitine. Although we cannot exclude the possibility that L-carnitine may be degradable to some extent by intestinal microflora in the large intestine,[6] the results in this study indicate that its absorption and any of its ^{14}C-labeled degradation products, if they exist, is insignificant in the large intestine in terms of the fraction which has disappeared from the intestinal lumen. According to a study by Rebouche *et al.* in the rat,[6] L-carnitine may be partly degraded in the large intestine preferentially to γ-butyrobetaine which is little absorbable than to trimethylamine *N*-oxide which is fairly absorbable, though a case was reported in human that the latter may be preferentially produced in some individuals.[14]

It has been suggested in the literature that L-carnitine may be partly acylated in the intestinal mucosa during absorption.[16—18] However, that may only be true at extremely low doses or concentrations, as reported in the rat for doses of 2 to 6 nmol/rat[17] and in the isolated enterocytes of the guinea pig at a concentration as low as 2 μM.[18] The suggestion of insignificant first-pass effect for L-carnitine from our preceding[11] and present studies is in agreement with earlier reports that: 1) L-carnitine does not undergo significant acylation in the rat intestinal mucosa[9]; 2) in the human, orally administered L-carnitine at a pharmacological dose mainly causes a rise in the concentration of unacylated L-carnitine in plasma with little efffect on the acylated forms that exist physiologically[19]; 3) radioactivity does not accumulate extensively in the liver after enteral administration of [^{3}H]L-carnitine to the rat.[16] Thus, quantitatively, the first-pass acylation of L-carnitine does not seem to be very significant in most situations.

It should also be noted that the biliary excretion of L-carnitine appears to be negligible, as reported in our preceding study.[11] In that study, only 0.005% of dose (or 0.006% of absorbed L-carnitine) was excreted in bile within the hour after administration into the closed loop of the rat small intestine (midgut) using a low dose (0.01 μmol/rat). This finding of negligible biliary excretion is in agreement with an earlier report by Gudjonsson *et al.*[16] who found only 0.34% of dose was excreted in bile within 4 h after enteral L-carnitine administration in the rat at 10 μmol/rat, which was closer to the high dose (100 μmol/rat) used here. They did not evaluate the fraction of L-carnitine dose absorbed in the same study, but noted that, for a shorter period of 2 h, it was 33%, that is about 100 times larger than the fraction excreted in bile during the longer period of 4 h. Thus, the biliary excretion of L-carnitine seems to be negligible over a wide range of doses.

Intestinal Transport *in Situ* To further confirm this *in vivo*, L-carnitine transport was examined in the closed loop *in situ*, comparing the small intestine (midgut) and the large intestine (colon) at a low concentration range of 0.005 to 0.05 mM and a high concentration range of 10 to 100 mM (Table 2). The former represents the approximate concentration range expected in the intestinal lumen after oral administration of a low dose of 0.05 mM of solution, and the latter represents that of a high dose of 100 mM of solution.

In the small intestine, the apparent membrane permeability clearances (CL_{app}) of L-carnitine were smaller by an order of magnitude in the high concentration range than in the low, in

Table 2. Intestinal Transport of L-Carnitine in the Closed Loop of Rat Intestine

Concentration (mM)	F_a (%)		CL_{app} (μl/min/cm)	
	SI	LI	SI	LI
0.005	49.3±2.6	—	2.28±0.17	—
0.01	52.6±2.8	3.1±2.1	2.50±0.20	0.054±0.036
0.05	36.6±5.1	—	1.54±0.26[a)]	—
10	13.8±2.3	3.1±1.4	0.25±0.05[b)]	0.052±0.024
100	11.1±0.3	—	0.20±0.01	—

Data are represented as the mean±S.E. (n=3). SI, Small intestine; LI, large intestine. F_a, Fraction absorbed in 30 min (0.005, 0.01 and 0.05 mM for SI) or 60 min (the others); CL_{app}, apparent membrane permeability clearance. Levels of statistical significance compared with the value at the next lower concentration: a) $p<0.05$; b) $p<0.01$.

agreement with earlier studies suggesting the involvement of saturable (carrier-mediated) transport as a source of dose dependency in gastrointestinal absorption *in vivo*.[7—10] Also in agreement with those earlier studies reporting the Michaelis constant (K_m) of 0.2 to 1.3 mM,[8—10] the CL_{app} was unchanged when concentration was raised from 0.005 to 0.01 mM, suggesting that those concentrations were far below K_m and the linearity of carrier-mediated transport was maintained. The CL_{app} value at 0.05 mM was significantly smaller than that at 0.01 mM but not significantly different from that at 0.005 mM. Thus, in the low concentration range, it was suggested that carrier-mediated transport is predominant and its linearity is largely maintained. The absorption efficiency of L-carnitine was, therefore, presumed to be maximized at the low dose. When the concentration was raised from 10 to 100 mM, the CL_{app} was unchanged at a level far lower than those in the low concentration range, suggesting that passive transport is predominant in the high concentration range. By 10 mM, the carrier-mediated transport is presumably saturated and its contribution is reduced to a negligible level, compared with passive transport. Therefore, the absorption efficiency of L-carnitine was presumed to be minimized at the high (or pharmacological) dose.

In the large intestine, L-carnitine transport was examined at 0.01 and 10 mM, which represent the low and high concentration ranges, respectively. The CL_{app} was unchanged when concentration was raised, and was at a level an order of magnitude lower than those for the higher concentrations (or for passive transport) in the small intestine. The low and concentration-independent permeability in a wide range of concentrations from the low to the high suggests the passive nature of L-carnitine transport in the large intestine. The smaller CL_{app} in the large intestine, compared with the CL_{app} of passive transport in the small intestine, may be at least partly due to the smaller anatomical surface area in the large intestine.[20]

In conclusion, orally administered L-carnitine is absorbed in a dose-dependent (saturable) manner in the small intestine but, regardless of dose, is not significantly absorbed in the large intestine presumably due to the extremely low apparent membrane permeability clearance associated with its passive nature of transport as demonstrated in the closed intestinal loop *in situ*. For each low and high dose, the fraction of L-carnitine dose absorbed (disappearing) from the gastrointestinal tract (small intestine) was in agreement with the bioavailability in our preceding study,[11] suggesting that the bioavailability is mainly defined by the saturable transport in the small intestine, without the involvement of significant first-pass degradation. These findings support our preceding kinetic modeling strategy assuming the small intestine to be the sole absorption site,[11] and should be of help in furthering studies to develop more efficient oral L-carnitine delivery strategies.

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