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Nasal absorption kinetics of human growth hormone enhanced by acylcarnitines in rats

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Abstract

Pharmacokinetic analysis revealed that acylcarnitines enhanced overall nasal absorption of human growth hormone (HGH) in rats. Acylcarnitines dramatically shortened onset time, shortened absorption-duration time, and efficiently delivered HGH. HGH administered intranasally with acylcarnitines appeared in the blood within 0.28–2.7 min, far more rapidly than the 11.7 min after intranasal administration of HGH without acylcarnitines (CTL) or the 9.8 min after subcutaneous administration (SC). Absorption-duration times of HGH enhanced by acylcarnitines were in the range of 2.1–3.8 h, which are shorter than those of CTL (7.5 h) or SC (9.8 h). Among acylcarnitines at 1%, lauroylcarnitine chloride (LCC) showed the greatest absolute bioavailability (17.4%), which is 0.73 times the absolute bioavailability value for SC (22.1%). LCC enhancement reached a plateau at a concentration of 0.1%. This nasal absorption enhancement would be suitable for pulsatile administration. A single dose toxicity study also suggested that LCC had similar systemic safety to L-carnitine at a high dose of 100 mg/kg in mice. Further, the potential safety of LCC was ten times greater than palmitoylcarnitine chloride.

Keywords: Human growth hormone; Nasal absorption enhancer; Acylcarnitines; Lauroylcarnitine; Pharmacokinetic analysis; Single dose toxicity

1. Introduction

The desirable therapeutic effect of human growth hormone (HGH) treatment on growth hormone (GH)-deficient children may best be obtained by pulsatile administration. While continuous infusion of HGH suppressed endogenous GH secretion in normal rats (Clark et al., 1988), pulsatile administration induced much greater growth than continuous infusion in chronically hypophysectomized rats (Clark et al., 1985). Furthermore, the growth depended on the frequency of pulsatile administration at the same dose of GH. It was fastest at the frequency of nine times/day which closely matches the normal pulse fre-

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quency in the body. Thus, chronopharmacologic studies have shown the optimum dosage regimen for HGH. Mimicking the physiological pulsatile secretory pattern of GH is important to obtain maximum therapeutic effect.

Currently, therapeutic HGH administration to GH-deficient children is by single daily subcutaneous or intramuscular dosing, which deviates greatly from physiological pulsatile secretion (Robinson, 1991). Furthermore, frequent repeated injection is physically and mentally stressful to patients. Alternative delivery systems employing non-parenteral routes of administration such as nasal delivery would therefore offer considerable advantages in patient compliance and may also allow for pulsatile administration of HGH for optimum therapeutic effect.

HGH is a large molecule (191-amino acid, 22000 daltons). Without an absorption enhancer, intranasal administration would yield poor therapeutic bioavailability. Formulation study of the nasal form must take a balance between enhancing efficacy and overall safety, especially concerning both local and systemic side effects. To date, a number of enhancers for HGH have been studied including polyoxyethylene 9-lauryl ether, sodium glycocholate and its derivatives (Daugherty et al., 1988), sodium tauro-24,25-dihydrofusidate (Baldwin et al., 1990; Lee et al., 1992; Hedin et al., 1993; Albertsson et al., 1995), amastatin, N-acetyl-L-cysteine, palmitoylcarnitine chloride (PCC; O'Hagan et al., 1990), lysophosphatidylcholine (Illum et al., 1990; O'Hagan et al., 1990; Fisher et al., 1991), and didecanoyl-L-α-phosphatidylcholine and α-cyclodextrin (Agerholm et al., 1994). Among these, local mucosal toxicity of polyoxyethylene 9-lauryl ether, deoxycholic acid, sodium tauro-24,25-dihydrofusidate, and lysophosphatidylcholine was demonstrated by measuring these compounds effects on mucociliary transport rate (Gizurarson et al., 1990), nasal morphology (Ennis et al., 1990), ciliary beat frequency (Hermens et al., 1990), release of nasal mucosal marker compounds (Marttin et al., 1995), or transepithelial electrical resistance (Lin and Shen, 1991). In contrast, the local mucosal safety of acylcarnitines has already been demonstrated. Examples include reversible in vivo absorption enhancement of the antibiotic cefoxitin by PCC (Fix et al., 1986), reversible in vitro transepithelial electrical resistance (TEER) of the colon carcinoma cell line Caco-2 by PCC (Raeissi and Borchardt, 1993; Hochman et al., 1994), reversible TEER of the colonic mucosa in vitro by PCC and lauroylcarnitine chloride (LCC; LeCluyse et al., 1993), little morphological change in the mucosa of Caco-2 cells by PCC (Fix et al., 1986; Hochman et al., 1994) and little change of the colonic mucosa in vivo by PCC and LCC (LeCluyse et al., 1993). This may be because acylcarnitines are endogenous amino acid-like compounds. Acylcarnitines have been previously investigated as possible nasal absorption enhancers for salmon calcitonin (SCT, 32-amino acid) by measuring pharmacological effects in rats (Kagatani et al., 1996). Results showed that LCC enhanced SCT absorption much better than PCC and was the strongest enhancer among acylcarnitines. LCC also showed a saturable and maximum enhancement at a concentration of 0.1%. It is of considerable interest to know whether these enhancing characteristics for SCT are universal for other peptides. It is also important to determine the enhanced absorption rate of peptides across the nasal epithelium by acylcarnitines.

In this study, blood HGH levels after intranasal administration of HGH to rats were determined by immunoradiometric assay. These data were analyzed kinetically how acylcarnitines enhance the nasal absorption of larger macromolecule HGH. Further, to aid in selecting the most clinically favorable enhancer among acylcarnitines for a nasal HGH formulation, their systemic toxicity in mice was also investigated.

2. Materials and methods

2.1. Chemicals

Lyophilized human growth hormone (HGH) (12 IU, 4 mg/vial) was obtained from Novo Nordisk Pharmaceutical Co., Ltd. (Denmark). It contains 60–80 mg glycine, 7.5–10 mg sodium bicarbonate, and 6–8 mg mannitol to stabilize HGH. L-Carnitine hydrochloride (CHC), DL-hexanoylcarnitine chloride (HCC), DL-octanoylcarnitinechloride (OCC), DL-lauroylcarnitine

chloride (LCC), myristoyl-DL-carnitine chloride (MCC), palmitoyl-DL-carnitine chloride (PCC) and sodium taurocholate (TRC) were purchased from Sigma (USA). All other reagents used were of reagent grade. The buffered HGH solution was adjusted to pH 7.4 with HCl after dissolving lyophilized HGH in water.

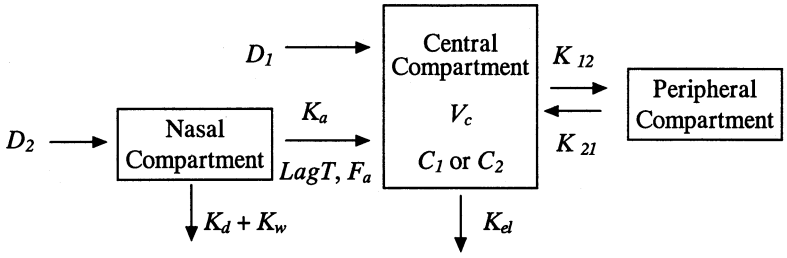
2.2. Animal experiments

All experiments were conducted in adherence to the principles of the Animal Ethical Committee of Yamanouchi Pharmaceutical. Male Wistar rats (257–291 g, 9 weeks) which fasted for 20 h before administration were used for pharmacokinetic studies. Anesthesia was induced by intraperitoneal injection of 50 mg/kg sodium pentobarbital (Nembutal®; Abbott Laboratories, USA) 10 min before administration and was maintained with additional injections of 40 mg/kg sodium pentobarbital. The nasopalatine was closed with a cyanoacrylate adhesive agent (Aron Alpha A®; Sankyo Co., Japan). A trachea tube was inserted to assist breathing. A buffered HGH solution (2.5 mg/0.4 ml/kg) without (control, CTL) or with an enhancer was administered separately through both nares with a micropipette within a 1-min period. HGH was injected intravenously (0.125 mg/0.8 ml/kg) into the jugular vein, or subcutaneously (2.5 mg/0.8 ml/kg) on the back. Blood (0.15 ml) was taken periodically from the jugular vein with a syringe containing water (0.15 ml) and stored at −20°C until assay. The HGH in a 100-μl sample was determined by immunoradiometric assay using a Sucrosep® GH kit (Boots-Celltech Diagnostics Limited, UK).

For the single dose toxicity study, male ICR mice (25.8–32.6 g, 6 weeks) given ad libitum access to feed were used. Mice were first weighed to baseline body weight values. Enhancers were dissolved in 0.9% NaCl solution (SAL) and administered intravenously (10–100 mg/10 ml/kg) for 28 days through the tail vein. The entire dose was injected within 1 min. SAL was used as a control. After administration, the body weight was measured again and the percent change in body weight was calculated.

2.3. Pharmacokinetic analysis

For pharmacokinetic analysis of the overall absorption behavior of HGH in rats, a two compartment model including a nasal (or subcutaneous) compartment (Model 1) and derived integrated equations (Eq. (1) and Eq. (2)) were used:



Model 1. Pharmacokinetic model of the overall nasal absorption behavior of HGH.

$$C_1 = \frac{D_1}{V_c}\left[\frac{(K_{21}-\alpha)}{(\beta-\alpha)}e^{-\alpha\times t} + \frac{(K_{21}-\beta)}{(\alpha-\beta)}e^{-\beta\times t}\right] \quad (1)$$

$$C_2 = \frac{F_a \times D_2 \times K_a}{V_c}\left[\frac{(K_{21}-K_a)}{(\alpha-K_a)\times(\beta-K_a)}e^{-K_a\times(t-LagT)} + \frac{(K_{21}-\alpha)}{(K_a-\alpha)\times(\beta-\alpha)}e^{-a\times(t-LagT)} + \frac{(K_{21}-\beta)}{(K_a-\beta)\times(\alpha-\beta)}e^{-\beta\times(t-LagT)}\right] \quad (2)$$

where V_c is the volume of the central compartment; α and β are the first-order macro-rate constants describing the disposition of the drug; K_{12} and K_{21} are the first-order rate constants for the transfer of drug between central and peripheral compartments; K_{el} is the first-order elimination

rate; C_1 and C_2 are the concentrations of drug administered intravenously and intranasally (or subcutaneously), respectively, D_1 and D_2 are the amounts of drug administered; $LagT$, F_a and K_a are the lag time, fraction absorbed and first-order absorption rate constant of nasal (or subcutaneous) absorption, respectively, and K_d (degradation rate) plus K_w (wash-out rate) is a mixed first-order rate constant. Absolute bioavailability (*Bioav*) was calculated as $F_a \times 100$. Absorption-duration time, the time to absorption of 95% of total possible HGH absorption ($T_{95\%}$) was calculated by $LagT - \ln(0.05)/K_a$.

Intravenous pharmacokinetic parameters were estimated by fitting Eq. (1) to the data from each rat. Mean values were then calculated. Using intravenous parameters of V_c, K_{12}, K_{21}, K_{el}, α and β, the intranasal (or subcutaneous) absorption pharmacokinetic parameters ($LagT$, F_a and K_a) were estimated by fitting Eq. (2) to individual data sets. Computation was carried out with the non-linear least-squares regression analysis program NONLIN84 (Metzler and Weiner, 1984) using Hartley and Levenberg's modification of the Gauss-Newton iteration algorithm on a VAX6210 digital computer (Digital Equipment Corp., USA).

2.4. Data analysis

All data are expressed as the mean ± S.E. To identify the source of any differences found, multiple comparisons of data were made by Scheffé's test using the SAS program (SAS/STAT® User's Guide, 1988).

3. Results and discussion

3.1. Nasal absorption kinetics of HGH with acylcarnitines in rats

In order to elucidate the overall absorption of HGH after intranasal administration with acylcarnitines in rats, pharmacokinetic analysis was performed according to the two compartment model outlined previously (Model 1) including nasal absorption pharmacokinetic parameters ($LagT$, F_a and K_a). HGH alone was administered intravenously (0.125 mg/kg) or subcutaneously (2.5 mg/kg). HGH either without an enhancer or with 1% OCC, 0.03–3% LCC, 1% PCC, or 1% TRC was administered intranasally (2.5 mg/kg). Blood HGH levels were determined by immunoradiometric assay. The solid lines in Figs. 1 and 2 represent the computer simulated curves. Calculated pharmacokinetic parameters are listed in Tables 1 and 2.

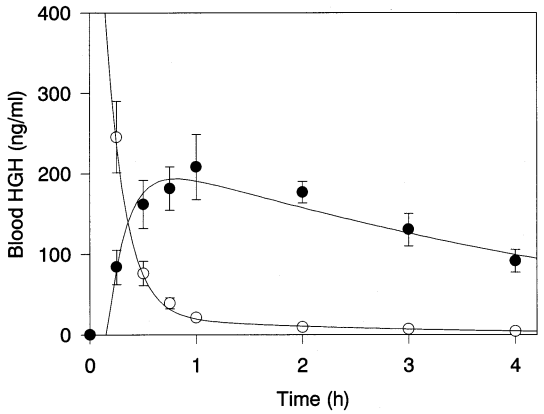


Fig. 1. Time course of blood HGH concentrations after intravenous (0.125 mg/kg) and subcutaneous (2.5 mg/kg) administration of HGH in rats. (○) intravenous ($r = 0.999$), (●) subcutaneous ($r = 0.985$). Solid curves were calculated from computer-fitted individual data of three or four rats. Each point represents the mean ± S.E.

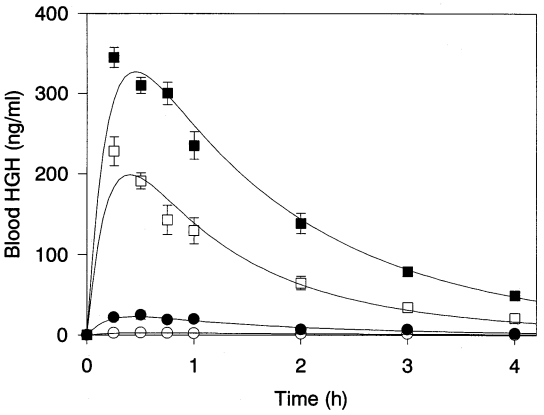


Fig. 2. Time course of blood HGH concentrations after intranasal (2.5 mg/kg) administration of HGH with acylcarnitines (1%) in rats. (○) CTL ($r = 0.900$), (●) OCC ($r = 0.982$), (□) PCC ($r = 0.965$), (■) LCC ($r = 0.979$). Solid curves were calculated from computer-fitted individual data of four rats. Each point represents the mean ± S.E.

Table 1
Pharmacokinetic parameters after intravenous administration of HGH (0.125 mg/kg) in rats

	AUC (ng·h/ml)	V_c (ml/kg)	CL (ml/h/kg)	α (1/h)	β (1/h)	K_{el} (1/h)	K_{12} (1/h)	K_{21} (1/h)	r
Mean	210	155	649	5.50	0.429	4.04	1.30	0.584	0.999
S.E.	31	24	105	0.51	0.058	0.27	0.29	0.086	0.000

Each parameter was calculated from computer-fitted individual data of four rats.

There were reasonably good fits between the predicted and observed points, with a correlation coefficient (r) of 0.999 for intravenous, 0.985 for subcutaneous, and between 0.900 and 0.996 for intranasal data, indicating that this pharmacokinetic analysis is suitable for determining the nasal absorption kinetic behavior of HGH.

Obtained pharmacokinetic parameters revealed that acylcarnitines have a short absorption-duration time and greatly enhance nasal absorption of HGH (Table 2). HGH appeared rapidly in the blood within 0.28–2.7 min ($LagT$) after intranasal administration with acylcarnitines. In contrast, HGH appeared at 11.7 min after intranasal administration of HGH without acylcarnitines (CTL) and at 9.8 min after subcutaneous administration (SC). The fact that total intranasal administration was completed within 1 min shows that absorption of HGH started just after administration. Absorption-duration times ($T_{95\%}$s) with acylcarnitines were in the range of 2.1–3.8 h, which were shorter than those of CTL (7.5 h) and SC (9.8 h). Furthermore, absolute bioavailabilities (*Bioav*s) of HGH enhanced by 0.1–3% LCC were in the range of 12.3–17.4%, which were close to that of SC (22.1%). *Bioav* with 1% LCC was 0.73 times that of SC. These findings suggest that intranasal administration of HGH highly enhanced by acylcarnitines may represent an alternative delivery system to non-parenteral systems. Furthermore, this administration could be applied to pulsatile delivery of HGH because these observed nasal HGH absorption kinetics of rapid appearance and short absorption-duration more closely resemble the endogenous pulsatile pattern of HGH (Robinson, 1991) than SC.

Structural dependency of absorption enhancing characteristics was examined by varying the number of carbon atoms in the acyl chain (n) as follows: $n=8$ (OCC), $n=12$ (LCC), and $n=16$ (PCC). LCC and PCC showed significant enhancement ($p<0.001$) in *Bioav* compared with CTL whereas OCC did not (Fig. 3). The dose-dependency of LCC on bioavailability was also studied, since it exhibited the best enhancing effect among acylcarnitines. *Bioav*s with 0.1–3% LCC were significantly greater than that with 0.03% ($p<0.001$, Fig. 4). The enhancement reached a plateau at 0.1% LCC and declined at 3%. Maximum enhancement was obtained at both 0.1 and 1%. Notably, this maximum and saturated enhancement of LCC is consistent with previous results on the nasal absorption of salmon calcitonin (SCT; Kagatani et al., 1996). LCC enhancement of both HGH (191-amino acid) and SCT (32-amino acid) reached a maximal plateau at a concentration of 0.1%, about three times as much as the critical micellar concentration. From the CMC values of acylcarnitines (OCC, 3%; LCC, 0.03%; PCC, 0.0004%), it was also suggested that micelle formation plays a key role in acylcarnitine enhancement of nasal HGH absorption as was seen with SCT. This enhancing ability of acylcarnitines as a function of acyl chain length may relate to the bell-shaped membrane perturbing capacity of acylcarnitines as previously reported (LeCluyse et al., 1993). In order to partition into the nasal mucosa and to perturb the lipid order, LCC may have optimal physical properties of size and lipophilicity. If the length of the acyl chain is longer, incorporation into the lipid bilayer of the membrane may be unfavorable.

Looking at the relationship between *Bioav* and *LagT*, these two parameters showed opposing trends. Fig. 3 shows effect of the number (n) of carbon atoms in the acyl chain of acylcarnitines at a concentration of 1% on nasal absorption of HGH in rats. LCC and PCC which gave the

Table 2
Pharmacokinetic parameters after intranasal or subcutaneous administration of HGH (0.25 mg/kg) in rats

Promoter (%)	*Bioav* (%)	K_a (1/h)	*LagT* (min)	$T_{95\%}$ (h)	*r*
CTL (—)					
Mean	0.4	0.411	11.74	7.48	0.900
S.E.	0.1	0.025	0.47	0.50	0.031
OCC (1)					
Mean	1.2	0.824	2.70	3.68	0.982
S.E.	0.1	0.126	0.28	0.22	0.007
LCC (0.03)					
Mean	1.9	0.820	5.33	3.74	0.954
S.E.	0.1	0.155	0.26	0.92	0.001
LCC (0.1)					
Mean	15.3	0.947	0.37	3.17	0.988
S.E.	1.8	0.108	0.10	0.36	0.003
LCC (1)					
Mean	17.4	0.793	0.28	3.78	0.979
S.E.	0.5	0.055	0.09	0.26	0.005
LCC (3)					
Mean	12.3	1.423	0.94	2.12	0.989
S.E.	0.8	0.109	0.13	0.16	0.002
PCC (1)					
Mean	8.5	1.061	0.30	2.83	0.965
S.E.	0.8	0.058	0.05	0.17	0.011
TRC (1)					
Mean	10.0	2.097	5.66	1.52	0.996
S.E.	1.4	0.498	1.22	0.27	0.001
SC (—)					
Mean	22.1	0.311	9.78	9.80	0.985
S.E.	1.4	0.029	0.96	0.90	0.006

Each parameter was calculated from computer-fitted individual data of three or four rats.

highest bioavailabilities showed the lowest *LagT*. Fig. 4 shows the effect of LCC concentration on values of *Bioav* and *LagT*. As the bioavailability of nasal HGH with LCC decreased from 17.4 to 1.9%, LagT increased from 0.28 to 5.33 min (Fig. 4). However, CTL exhibited a minimum *Bioav* of 0.4% and a maximum *LagT* of 11.7 min as shown in Table 2. *LagT* (5.7 min) of 1% TRC was longer than those of 3% LCC (0.94 min) or 1% PCC (0.30 min), which had similar *Bioav* to TRC. Further, the decreased $T_{95\%}$ of TRC was different to those of acylcarnitines. Thus, TRC and acylcarnitines showed different enhancement behaviors on the nasal absorption of HGH. Although the mechanism of this enhancement has not been fully clarified, the results of this study clearly show that acylcarnitines react very rapidly with the nasal absorption site and efficiently deliver HGH to the blood. The paracellular transport mechanism of PCC in a Caco-2 cell line in vitro was recently described (Hochman et al., 1994). PCC contributed to a rapid drop in transepithelial electrical resistance (TEER) within the first minute after administration and a prolongation of TEER for approximately 20 min. This effect was reversible after the removal of PCC.

Interestingly, PCC produced significant structural alterations to tight junctions, which were different to those caused by other tight junction disrupting agents. Dose dependency of acylcar-

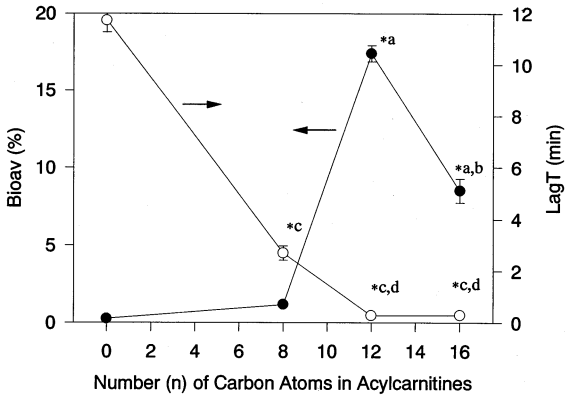


Fig. 3. Effect of the number (n) of carbon atoms in the acyl chain of acylcarnitines (1%) on nasal absorption of HGH (2.5 mg/kg) in rats. (○) *LagT*, (●) *Bioav*. Each point represents the mean ± S.E. of four rats. (*a) Significantly different from $n = 0$ (CTL) and $n = 8$ (OCC), (*b) from $n = 12$ (LCC), (*c) from $n = 0$, and (*d) from $n = 8$ by Scheffé's multiple comparison ($p < 0.001$).

nitines on TEER and calcein flux in colonic mucosa in vitro was also reported (LeCluyse et al., 1993). With increased concentration of LCC, the rate of TEER decrease became more pronounced and the lag time of calcein transport decreased. This fact seems to reflect pharmacokinetic results of this study that *LagT* decreased with an increase of LCC concentration from 0.03 to 1% (Table 2). However, the absorption kinetic parameter at 3% LCC was

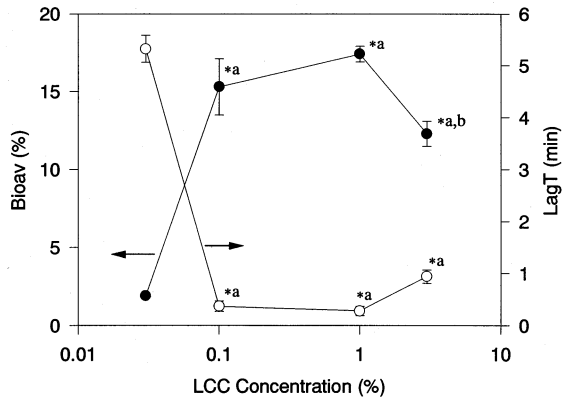


Fig. 4. Nasal HGH (2.5 mg/kg) absorption in rats as a function of LCC concentration. (○) *LagT*, (●) *Bioav*. Each point represents the mean ± S.E. of four rats. (*a) Significantly different from 0.03% ($p < 0.001$) and (*b) from 1% ($p < 0.05$) by Scheffe's multiple comparison.

different from those at lower LCC concentrations. At 3% LCC, K_a showed 1.5–1.8 times greater values over those at lower concentrations. These pharmacokinetic results reinforce the proposition that acylcarnitine may enhance drug absorption via two different mechanisms, as follows: a low concentration-dependent absorption via the paracellular route and a high concentration-dependent absorption via breaks or gaps appearing in the epithelium, producing increased drug absorption rate compared with that at lower concentrations (LeCluyse et al., 1993).

3.2. Single dose toxic study of acylcarnitines in mice

In order to compare the potential safety of acylcarnitines, single dose toxicity after intravenous administration at high doses (30–100 mg/kg) was studied in mice. Mice receiving 100 mg/kg of HCC ($n = 6$), OCC ($n = 8$), or LCC ($n = 12$) revealed similar weight gain to those receiving CHC (L-carnitine hydrochloride, $n = 0$) and SAL (saline injection control). However MCC ($n = 14$) and PCC ($n = 16$) showed lethal toxicity with death rates of 67 and 100%, respectively (Fig. 5). When the PCC dose was decreased from 100 to 10 mg/kg, growth reached levels obtained with acylcarnitines ($n < 14$) at 100 mg/kg. The potential safety of LCC or shorter-chain acylcarnitines were nearly ten times greater than that of PCC.

It is worth noting that this superior systemic safety of LCC to that of PCC coincides with the superiority of LCC to PCC in rapid recovery of transepithelial electrical resistance and in the absence of mucosal damage (LeCluyse et al., 1993). LCC also induces no morphological damage in nasal mucosa, and its enhancement of nasal SCT absorption is reversible (unpublished data). Taken together these results suggest that LCC is a more promising candidate than PCC for safely enhancing the nasal absorption of HGH.

4. Conclusions

Results of pharmacokinetic analysis suggest that intranasal absorption of HGH is enhanced signifi-

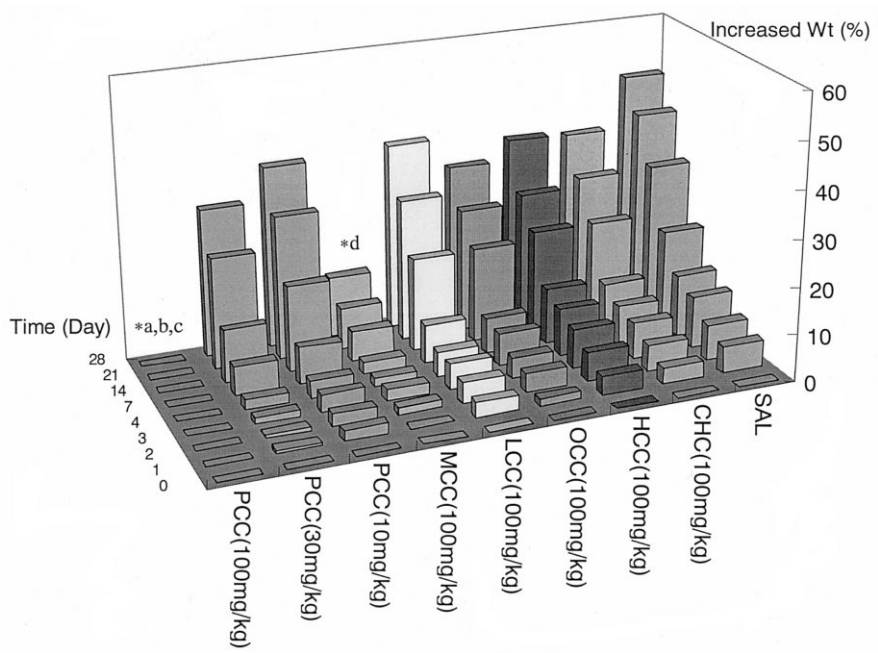


Fig. 5. Effect of acylcarnitines on the growth of mice after intravenous injection. Each point represents the mean ± S.E. of three or four mice. Two out of three or all of the three mice died after intravenous injection of 100 mg/kg MCC or 100 mg/kg PCC, respectively. (*a) Significantly different from SAL ($p < 0.01$), (*b) from 100 mg/kg LCC ($p < 0.05$), (*c) from 10 mg/kg PCC ($p = 0.059$), and (*d) from SAL ($p = 0.073$) by Scheffé's multiple comparison.

cantly by acylcarnitines and has a potential as a pulsatile delivery therapy which matches physiological secretion of HGH. Among acylcarnitines, LCC has the greatest enhancing ability on nasal HGH absorption and better systemic safety than other acylcarnitines.

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