Evidence for Dissolution Rate-Limited Absorption of COL-3, a Matrix Metalloproteinase Inhibitor, Leading to the Irregular Absorption Profile in Rats after Oral Administration

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Purpose. This study was undertaken to elucidate the underlying mechanism of the irregular absorption profiles of COL-3, a matrix metalloproteinase inhibitor, with a double- or plateau-peak concentration after a single oral dose administration of COL-3 suspension to rats.

Methods. The gastrointestinal absorption profiles of COL-3 in rats were assessed by comparing serum drug concentration curves after the following various modes of drug administration: oral and intraduodenal doses, oral doses of COL-3 in fine and coarse suspensions, intraduodenal dosing to the bile-duct intact and cannulated (BDC) rats, and oral doses with and without food. In addition, the biliary excretion of COL-3 in the BDC rats was examined.

Results. Neither variable gastric emptying nor enterohepatic recycling was the source of the irregular gastrointestinal absorption of COL-3 in rats. Reduction in particle size, presence of food and endogenous bile emerged as the determinants of the oral absorption of COL-3 by enhancing the dissolution of the solid drug in the gastrointestinal fluids. Flip-flop of the absorption and elimination rate constants was noted only for COL-3 after intraduodenal administration of the coarse suspension to the BDC rats with the bile flow diverged out of the body.

Conclusions. Variability in dissolution rate-limited absorption was the main cause of the irregular absorption of COL-3 after oral administration of its solid dosage form.

KEY WORDS: COL-3; matrix metalloproteinase inhibitor; irregular absorption; double- or plateau-peak phenomenon; dissolution rate-limited absorption; flip-flop situation.

INTRODUCTION

COL-3, 6-deoxy-6-demethyl-4-dedimethylaminotetracycline, also known as CMT-3 (Metastat®, PA, USA), is a chemically modified tetracycline with antimicrobial activity eliminated. COL-3 exhibits *in vitro* and *in vivo* activity as a matrix metalloproteinase inhibitor (1,2). The matrix metalloproteinases (MMPs) belong to a family of Zn^{2+}-dependent proteinases, which proteolytically degrade the extracellular matrix. Degradation of this matrix, especially the basement membrane, is essential for tumor cell invasion, metastasis, growth, and angiogenesis (3,4). Pre-clinical (2,5,6) and phase I clinical studies (7) have demonstrated that COL-3 has both antitumor and anti-metastasis activity. The mechanisms of action for COL-3 include the inhibition of MMPs (2,5), induction of apoptosis (8), and inhibition of cell proliferation (2,8).

COL-3 is practically insoluble in water (approximately 0.01 mg/mL), and readily soluble in organic solvents, such as methanol, polyethylene glycol, and benzyl alcohol. The solubility of COL-3 increases, but its stability decreases, with increasing pH. The pH of its maximum stability is below 4 (9). Due to its high hydrophobicity as well as its instability in alkaline condition, COL-3 has been formulated as a capsule or a suspension for oral administration in clinical and animal studies (5,7).

In our preliminary study, there is an irregular absorption profile of COL-3 with double- or plateau-peak concentration following a single oral administration of COL-3 suspension to rats. The double-peak phenomenon has also been observed in another study (Liu *et al.*, unpublished data, 1999). In the phase I clinical trial of oral COL-3 in patients with refractory metastatic cancer, COL-3 has been found to possess a long and varied terminal half-life value between patients (median, 56.7 h; range, 23.7-144.4 h), besides a large inter-individual variation in time of occurrence for peak concentration (median, 6.0 h; range, 2.0-48.1 h) (7). Details of the absorption and disposition processes of COL-3 in the literature are still lacking.

The purpose of the present study was thus to investigate the underlying mechanisms of the irregular absorption for COL-3 following a single oral administration of COL-3 suspension to the rats and to provide an explanation to the unusual long and varied terminal half-life of COL-3 observed in patients.

Several mechanisms have been proposed to account for the double-peak phenomenon including variable gastric emptying (10,11), enterohepatic recycling (EHC) (12,13), and discontinued or variable absorption rate constant along the gastrointestinal tract (14,15). In order to examine the effect of variable gastric emptying on the formation of double peaks, COL-3 was administered into the duodenum of the rats. In order to evaluate the role of EHC in the absorption and disposition of COL-3, the biliary excretion of COL-3 and its possible phase II metabolites were determined. Since COL-3 is highly hydrophobic, in order to clarify the likely dissolution-rate limited absorption of COL-3 following oral administration of the solid drug the effects of formulation, food, and endogenous bile on the gastrointestinal absorption of COL-3 were thus evaluated. In addition, a solution for intravenous injection of COL-3 was developed and the disposition of COL-3 following its intravenous injection was investigated.

MATERIALS AND METHODS

Chemicals and Reagents

COL-3 was a gift from CollaGenex Pharmaceuticals, Inc. (Newtown, Pa.; USA). Carboxylmethyl cellulose sodium

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ABBREVIATIONS: BDC, Bile-duct cannulated; EHC, Enterohepatic recycling; CMC, Carboxylmethyl cellulose sodium; C_{max}, Maximum (peak) serum concentration; t_{max}, Peak time; AUC_{0-t}, Total area under the serum concentration-time curve from time zero to the last measurable time point; $AUC_{0-\infty}$, Total area under the serum concentration-time curve from time zero to infinity; λ_z, Terminal rate constant; $t_{1/2, \lambda z}$, Terminal half-life; CL, Total clearance; V_Z, Apparent volume of distribution based on the terminal phase; F, Absolute bioavailability.

(CMC), N-methyl-2-pyrrolidinone, polyethylene glycol 400 (PEG 400), phenolphthalein β-glucuronic acid, β-glucuronidase (Type B-3: from bovine liver, 4000 U/mg) and sulfatase (Type V: from Limpets, 7.6 U/mg) were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Midazolam and Hypnorm® were provided by Animal Holding Unit (National University of Singapore). All other chemicals and solvents used in this study were of analytical grade or high performance liquid chromatographic (HPLC) quality.

Oral Formulations of COL-3

Two formulations, COL-3 coarse and fine suspensions, recommended by CollaGenex Pharmaceuticals, Inc. (Newtown; Pa., U.S.A.), were used in the present study. The former was prepared by suspending COL-3 in 2% (w/v) CMC to obtain COL-3 concentration of 5 mg/mL, as described in the previous pre-clinical study (Liu *et al.*, unpublished data, 1999); while the latter was prepared using the method developed by CollaGenex Pharmaceuticals, Inc. (Newtown, Pa., U.S.A.). Briefly, 30 mg of COL-3 was dissolved in 0.1 mL of N-methyl-2-pyrrolidinone (containing 5% ethanol) as a stocking solution; close to the time of administration, 2 mL of distilled water and 4 mL of 2%(w/v) CMC were added to the stocking solution with constant stirring, and a fine suspension with COL-3 concentration of 5 mg/mL was obtained. The particle sizes of COL-3 coarse and fine suspensions were determined using a hemocytometer slide in large magnification (44Xobjective) under the microscopic analysis (Olympus, CHS). For the former, particles were irregular shape with the size range of 10–66 μm (39.6 ± 25.3 μm, n = 100), whereas for the latter, particles were spherical with the diameter range of 1-5 μm (2.68 ± 1.38 μm, n = 100).

Intravenous Injection Formulation of COL-3

A COL-3 solution was freshly prepared prior to its intravenous injection to the rats. 15 mg of COL-3 was dissolved in 2.4 mL of PEG-400, followed by mixing with 3.6 mL of phosphate buffer (0.1 M, pH 7.6). A crystalline yellow solution with COL-3 concentration of 2.5 mg/mL was obtained.

Animals

Male Sprague-Dawley (SD) rats (180–260 g) were obtained from Laboratory Animal Center (National University of Singapore, Singapore), and housed in temperature-controlled room (25 ± 1°C) with a 12-h light-dark cycle. The animals had free access to food (standard mouse pellets) and water *ad libitum* during the experiment, unless stated otherwise. They were randomly assigned into six groups with 6–9 rats each group. Group A (9 rats) received COL-3 coarse suspension orally. Group B (6 rats) received COL-3 fine suspension orally. Group C (6 rats) was given COL-3 coarse suspension intraduodenally. Group D (8 bile-duct cannulated (BDC) rats) was given COL-3 coarse suspension intraduodenally. The group A-D rats were allowed access to food before and after dosing. Food effect on the absorption of COL-3 was thus assumed to be at least similar among these groups. Group E (7 rats) was fasted overnight prior to the oral administration of COL-3 coarse suspension and allowed access to food 1 h postdose. Group F (6 rats) was intravenously injected COL-3 solution after overnight fasting. The group A-E rats were given COL-3 at the dose of 30 mg/kg, while the group F rats at that of 10 mg/kg. The research adhered to the principles of laboratory animal care (NIH publication #85-23, revised 1985).

Gastrointestinal Absorption of COL-3

The Effect of Formulation on the Absorption of COL-3

The COL-3 serum data obtained from the group A and B (receiving the coarse and fine suspensions, respectively) were compared.

The Effect of Bile on the Absorption of COL-3

On the day of the experiment, the group C rats were anesthetized by intraperitoneal injection of a mixture of midazolam/hypnorm®/water (1:1:2), 2.5 mL/kg. All surgery procedures were done using half-aseptic technique. The duodenum was exposed by laparotomy. The COL-3 coarse suspension was injected into the duodenum directly using a 2-mL syringe with 23-gauge needle. The abdominal incision was thus closed with silk sutures. The whole procedure lasted about 5 min. The rats were recovered from anesthesia after half an hour and had access to food and water *ad libitum*.

For the group D, rats were anesthetized as described above. The duodenum and bile duct were exposed by laparotomy. The exposed areas were covered with gauze moistened with saline to prevent dehydration and any damage to the tissue. The bile duct was cannulated according to the method of Wang and Reuning (16). Briefly, the bile duct was isolated, and an incision was made in the duct and a polyethylene catheter (PE 10, I.D. 0.28 mm, O.D. 0.61 mm) was inserted toward the liver (opposite to the direction of bile flow). The catheter was fixed and exteriorized. After about 200 μl of blank bile was collected, the COL-3 coarse suspension was injected to the rat duodenum as described above. The abdominal incision was then closed with silk sutures. The whole procedure lasted about 15 min. The rats were recovered from anesthesia after half an hour, and were allowed water and food *ad libitum*.

The Effect of Food on the Absorption of COL-3

The COL-3 serum data obtained from the group A (fed rats) and group E (fasted rats) were compared.

Intravenous Injection of COL-3

For the group F rats, the right femoral vein was cannulated with a polyethylene cannula (PE 10, I.D. 0.28 mm, O.D. 0.61 mm) for intravenous administration of COL-3. The rats were allowed to recover and then were fasted overnight prior to the dosing. The COL-3 solution (1 mL) was injected into the femoral vein at the dose of 10 mg/kg.

Determination of COL-3 Concentration in Serum

Blood Samples

The rats were under diethyl ester anesthesia while blood samples were taken, using orbital bleeding technique, prior to and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 30, 35, and 48 h after COL-3 administration. About 400 μl of blood obtained without anticoagulation was centrifuged (10 min at 3000 rpm, at

10°C) to separate serum. Serum samples obtained were then stored at –20°C until analyzed.

HPLC Assay

COL-3 concentration in serum was determined using the reverse-phase high-performance liquid chromatography method of Rudek *et al* (17) with some modifications. Briefly, 150 μl of serum sample was deproteinized by adding 300 μl of acetonitrile-methanol precipitant (acetonitrile: methanol: 0.5 M oxalic acid = 70:20:10, v/v). The mixture was vortex-mixed for 30 s, allowed to stand at room temperature for at least 10 min and centrifuged (17000 rpm, 8 min, 4°C). The supernatant was collected and an aliquot of 20 μl was subject to the HPLC assay. The SHIMADZU HPLC system (SHIMADZU Corp.; Kyoto, Japan) used was composed of an SCL-10Avp system controller, a LC-10ATvp pump, a DGU-14A degasser, a SIL-10ADvp autosampler, and an SPD-10Avp UV-VIS detector. Chromatography separation was conducted on a Waters XTerra™ RP_{18} column (150 × 4.6 mm I.D., particle size 5 μm) with Sentry™ guard column (XTerra™ RP_{18}, 20 × 3.9 mm I.D., particle size 5 μm) (Waters Cop., Milford; Mass., U.S.A.). The mobile phase which consisted of acetonitrile, methanol, and 0.01M oxalic acid (pH 2.0) (40:10:50, v/v) was delivered at a flow-rate of 1.0 mL/min. The detection wavelength was 350 nm. Peak recording and integration was performed using SHIMADZU CLASS-VP data management system. The limit of quantification (LOQ) for COL-3 in rat serum was determined to be 40 ng/mL. The accuracies at the concentration of 100, 1200, and 12000 ng/mL ranged within 99.7–104.0%. The within-day and between-day precision for COL-3 at the three quality control concentrations varied from 2.39–9.45% and 0.23–3.03%, respectively.

The Bile Excretion of COL-3 and Possible Phase II Metabolites

Bile Samples

Total bile was collected during the entire 48-h study period at an interval of 1 h from 0–12 h, and at the interval of 12–24, 24–35, and 35–48 h after administration of COL-3 to the group D rats. The bile samples were then kept at –20°C until analyzed. Each bile sample was subject to two treatment: one was with metabolic enzyme incubation in order to determine the total concentration of COL-3 and its possible phase II metabolites, whereas the other was without incubation in order to determine the unchanged COL-3.

Determination of the Total Concentration of COL-3 and Its Phase II Metabolites in Bile

To identify any possible phase II metabolites of COL-3, an aliquot (200 μl) of the bile sample was incubated with β-glucuronidase and sulfatase, prior to the HPLC assay. Briefly, the metabolic enzyme solutions were freshly prepared by dissolving appropriate amount of β-glucuronidase (Sigma type B-3) and sulfatase (Sigma type V) in 0.1 M NaCl. To each of the bile samples, 200 μl of 0.2 M sodium acetate (pH 5.0) was added, followed by 800 U of β-glucuronidase or 30 U of sulfatase. The mixtures were then incubated overnight in a shaking water bath at 37°C. The control incubations (no enzyme added) as well as the test incubations verifying enzyme viability (with phenolphthalein glucuronide added) were concurrently run. The COL-3 concentration in bile was determined using the HPLC assay method as described above.

Data Analysis

Irregular Absorption Behavior

An irregular absorption behavior was identified according to the following reported criteria (18): (1) Serum concentration profiles were considered to exhibit a double-peak phenomenon whenever between the two peaks there were at least two serum levels lower than the two peak maxima or the trough serum level was at least 10% lower than the smaller peak maximum, and (2) Serum concentration profiles were considered to exhibit a plateau-peak phenomenon whenever serum levels were within 25% of the peak maximum for at least two sampling time intervals (4 h).

Pharmacokinetic Parameters

The peak serum concentration(s) ($C_{max,\ 1}$, $C_{max,\ 2}$) and the time(s) of occurrence for peak concentration ($t_{max,\ 1}$, $t_{max,\ 2}$) were obtained by visual inspection of the serum concentration-time curves after the extravascular (oral or intraduodenal) dose. The total area under the serum concentration-time curve from time zero to the last measurable time point ($AUC_{0\text{-}t}$) was calculated using the linear and logarithmic trapezoidal methods for ascending and descending serum concentrations (the extravascular data), respectively, while that using only the logarithmic trapezoidal method for the intravenous data. The total area under the serum concentration-time curve from time zero to infinity ($AUC_{0\text{-}\infty}$) was calculated as the sum of $AUC_{0\text{-}t}$ and the extrapolated area, which was calculated by the last measurable serum concentration divided by the terminal rate constant (λ_z), where λ_z was estimated using the terminal log-linear phase of the plasma concentration-time curve. The terminal half-life ($t_{1/2\ \lambda z}$) was calculated as $0.693/\lambda_z$. The absolute bioavailability (F) was calculated as the ratio of extravascular to intravenous dose-normalized mean $AUC_{0\text{-}\infty}$ values. The total serum clearance (CL) was calculated as F *Dose/$AUC_{0\text{-}\infty}$. The apparent volume of distribution based on the terminal phase (V_z) was calculated as CL/λ_z.

Statistical Analyses

Statistical analyses were performed using SPSS 10.0 (SPSS Inc., USA). Data were expressed as mean ± standard deviation (SD). Comparisons of means for each of the pharmacokinetic parameters among the six experimental groups (A-F) were performed using one-way analysis of variance (ANOVA). The Levene test was used to test for any violation of the equal variance assumption. The post hoc multiple comparisons were performed using the Scheffé test for equal variance and the Tamahane's test for unequal variance. A value of $p < 0.05$ was taken to indicate statistical significance.

RESULTS

Gastrointestinal Absorption of COL-3

Double- or Plateau-Peak Phenomenon

Fig 1 illustrates the typical time-profiles of serum concentration of COL-3 following various modes of administra-

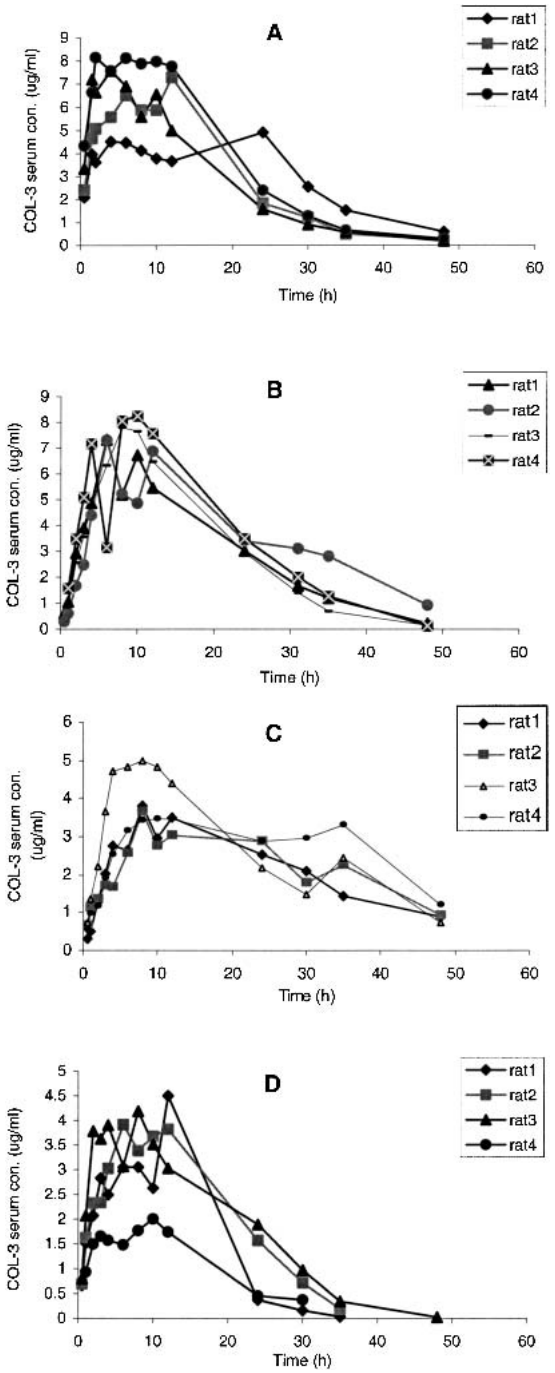

Fig. 1. The typical individual serum concentration-time profiles of COL-3 following (A) oral administration of the coarse suspension to the fed rats; (B) intraduodenal administration of the coarse suspension to the bile-duct intact fed rats; (C) intraduodenal administration of the coarse suspension to the bile-duct cannulated fed rats; and (D) oral administration of the coarse suspension to the fasted rats. The COL-3 was given at the dose of 30 mg/kg.

tion of the coarse suspension (30 mg/kg) in rats. An erratic or irregular absorption profile of COL-3 with either a double- or plateau-peak concentration was observed in each of the rats studied. The first peak was achieved at 2–10 h (i.e., $t_{max,1}$), while the second one occurred at 10–35 h (i.e., $t_{max,2}$) after COL-3 administration. The two peak concentrations appeared to have a similar mean value (Table 1). Furthermore, the double-peak phenomena vanished from, while the plateau-peak phenomena prevailed in, the mean serum concentration-time profiles of COL-3 as shown in Fig 2 A-D, due likely to the wide inter-individual variations of $t_{max,1}$ and $t_{max,2}$.

The Effect of Gastric Emptying on the Absorption of COL-3

The double- or plateau-peak phenomenon remained present in the individual serum profile after intraduodenal administration of the coarse suspension (Fig 1B). The mean serum profile of COL-3 after intraduodenal administration (Group C) was similar to that after oral administration (Group A) (Fig 2A). There were no significant differences in $C_{max,1}$, $C_{max,2,}$ $t_{max,1}$, $t_{max,2}$, $AUC_{0\text{-}48}$, $AUC_{0\text{-}\infty}$ and $t_{1/2,\lambda z}$ between the two groups (Table 1).

The Effect of Formulation on the Absorption of COL-3

The mean serum concentration-time profiles of COL-3 following oral administration of both coarse and fine suspensions were distinctly different (Fig 2B). The $C_{max,1}$, $C_{max,2}$, $AUC_{0\text{-}48}$ and $AUC_{0\text{-}\infty}$ for the fine suspension (Group B) were statistically significant higher than those obtained for the coarse suspension (Group A) (Table 1). No significant differences were found in $t_{max,1}$, $t_{max,2}$ and $t_{1/2,\lambda z}$ between the two formulations.

The Effect of Bile on the Absorption of COL-3

As shown in Fig 2C, the mean serum concentration-time profile of COL-3 following intraduodenal administration in the BDC rats (Group D) was markedly different from that in the intact rats (Group C). The observed $C_{max,1}$, $C_{max,2,}$ and $AUC_{0\text{-}48}$ values for the former were significantly lower than those for the latter. No significant differences in $t_{max,1}$, t_{max2}, $AUC_{0\text{-}\infty}$ were found between the two groups. The terminal $t_{1/2,\lambda z}$ value obtained from the BDC rats was prolonged significantly (about 2.7-fold) compared with the intact rats.

The Effect of Food on the Absorption of COL-3

As demonstrated in Fig 2D, the mean COL-3 serum concentrations in the fed rats (Group A) were significantly higher than those in the fasted rats (Group E) at all time points. Oral administration of the coarse suspension in the presence of food resulted in a 0.9- to 1.3-fold increase in C_{max1}, C_{max2}, $AUC_{0\text{-}48,}$ and $AUC_{0\text{-}\infty}$. No statistically differences in $t_{max,1}$, t_{max2} and $t_{1/2,\lambda z}$ were found between the two groups.

Biliary Excretion of COL-3

Following intraduodenal administration of COL-3 to the BDC rats (Group D), 1.28 ± 0.54% and 2.03 ± 0.63% of the administered dose (n = 8) were recovered from the bile as the unchanged COL-3 and the total of COL-3 and its glucuronide conjugate, respectively, over 0-48 h collection period. No sulfatate conjugate of COL-3 was detectable in bile.

Intravenous Injection of COL-3

Figure 2E shows the mean serum concentration-time profile of COL-3 following its intravenous injection to the fasted rats (Group F). The mean serum level of COL-3 ap-

Table I. Estimates of Pharmacokinetic Parameters for COL-3[a]

Parameters	Oral administration to fed rats (30mg/kg)		Intraduodenal administration of coarse suspension (30 mg/kg) to		Oral administration of coarse suspension (30mg/kg) to fasted rats (E) (N = 7)	Intravenous injection of solution (10mg/kg) to fasted rats (F) (N = 6)
	Coarse suspension (A) (N = 9)	Fine suspension (B) (N = 6)	Bile-duct intact fed rats (C) (N = 6)	Bile-duct cannulated fed rats (D) (N = 8)		
$C_{max,1}$ (μg/ml)	7.25 ± 1.68 *BDE	9.42 ± 1.46 *ACDE	7.24 ± 0.36 *BDE	4.11 ± 0.76 *ABC	3.52 ± 0.94 *ABC	8.40 ± 0.80[c]
$C_{max,2}$ (μg/ml)	7.09 ± 1.79 *BDE	9.86 ± 0.77 *ACDE	7.29 ± 0.84 *BDE	2.86 ± 0.58 *ABC	3.78 ± 0.99 *ABC	—
	(n = 8)[b]	(n = 4)[b]	(n = 3)[b]	(n = 6)[b]	(n = 6)[b]	
$T_{max,1}$ (h)	5.25 ± 1.83	8.00 ± 3.35	5.67 ± 1.50	8.50 ± 2.78	5.28 ± 2.06	—
$T_{max,2}$ (h)	12.00 ± 4.90 (n = 8)[b]	13.33 ± 5.46 (n = 4)[b]	10.67 ± 1.15 (n = 3)[b]	23.17 ± 12.98 (n = 6)[b]	10.33 ± 1.50 (n = 6)[b]	—
AUC_{0-48} (μg h/ml)	145.9 ± 31.22 *BDE	233.6 ± 24.51 *ACDE	146.4 ± 15.43 *BDE	110.5 ± 22.81 *ABCE	63.64 ± 17.64 *ABCD	78.56 ± 8.18[d] *ACDE
$AUC_{0-\infty}$ (μg h/ml)	147.3 ± 31.51 *BE	236.9 ± 25.04 *ACDE	148.5 ± 16.59 *BE	130.0 ± 29.55 *BE	64.56 ± 17.77 *ABCD	79.05 ± 8.41[d] *ACDE
CL (l/h/kg)	0.131 ± 0.024	0.128 ± 0.014	0.128 ± 0.013	0.135 ± 0.044	0.134 ± 0.039	0.128 ± 0.014
λ_z (h^{-1})	0.124 ± 0.025 *D	0.118 ± 0.011 *D	0.117 ± 0.014 *D	0.044 ± 0.004 *ABCEF	0.120 ± 0.026 *D	0.121 ± 0.018 *D
$T_{1/2,\lambda z}$ (h)	5.77 ± 1.19 *D	5.92 ± 0.58 *D	6.01 ± 0.70 *D	15.91 ± 1.43 *ABCEF	6.05 ± 1.45 *D	5.81 ± 0.76 *D
V_z (L/kg)	1.08 ± 0.24	1.10 ± 0.19	1.12 ± 0.19	3.05 ± 0.76 *ABCEF	1.21 ± 0.599	1.10 ± 0.17
F	62%	100%	63%	55%	27%	—

[a] Results are given as the mean ± SD.
[b] The number of rats with the occurrence of the second peak.
[c] The initial serum concentration of COL-3 after intravenous injection.
[d] The comparisons were based on the dose-adjusted area under the curve.
* Statistically different from groups ($p < 0.05$).

peared to fall mono-exponentially with its elimination half-life of 5.81 ± 0.76 h (n = 6). The values of V_z and CL estimated were 1.10 ± 0.17 l/kg (n = 6) and 0.128 ± 0.014 l/h/kg (n = 6), respectively.

DISCUSSION

Enterohepatic recycling (EHC) has been reported to be responsible for the double-peak phenomena of many drugs, including morphine (13), glycyrrhizin (12), and flurbiprofen (19). In the present study, EHC could be excluded as the main mechanism to account for the double-peak concentration and long terminal half-life of COL-3, since COL-3 manifested a very low biliary excretion. The poor biliary excretion of COL-3 might be due to its relatively small molecular weight (371.35) and extreme lipophilicity because for extensive biliary excretion to occur, molecules should possess a large molecular weight (> MW of 400–500) and a strong polar group (20). In addition, the presence of a double-peak concentration following single intraduodenal administration of COL-3 to the BDC rats with the bile flow diverged out of the body (Fig 1C) further supports that EHC played little role in the appearance of the second peak in the serum concentration-time profile of COL-3.

Delayed or variable gastric emptying has been proposed as the mechanism contributing to the double-peak concentration of some highly hydrophilic drugs, such as cimetidine (10) and celiprolol (11). However, for a highly hydrophobic drug like COL-3, gastric emptying is unlikely to be a rate-limiting step to its absorption. As shown in Fig 1B, variable gastric emptying could be ruled out since the double-peak concentration still prevailed after intraduodenal administration; meanwhile, the rate and extent of absorption of COL-3 following intraduodenal administration (Group C) were similar to those following oral administration (Group A) (Table 1). This observation is in close agreement with the finding of Paulson *et al.* (21) on celecoxib, another highly hydrophobic drug that exhibits the double-peak concentration in dogs following the same modes of administration with a similar extent of absorption.

In general, the absorption of a solid drug from the gastrointestinal (GI) tract involves four steps: 1) gastric emptying and small intestinal transit flow to deliver the drug to its absorption site; 2) dissolution of the solid drug into solution available for absorption; 3) permeation of the dissolved drug through the gastrointestinal membrane; and 4) moving the drug away from the site of absorption into the general circulation (22). For poorly water-soluble drugs, dissolution (step 2) is always the rate-limiting process to the absorption. In the present study, the significant effects of formulation, endogenous bile, and food on the absorption of COL-3 appeared to provide some evidence of dissolution rate-limited absorption for COL-3.

There was a marked difference in the serum profile of COL-3 between the fine suspension (mean particle size, 3μm) and the coarse suspension (mean particle size, 40μm) (Fig

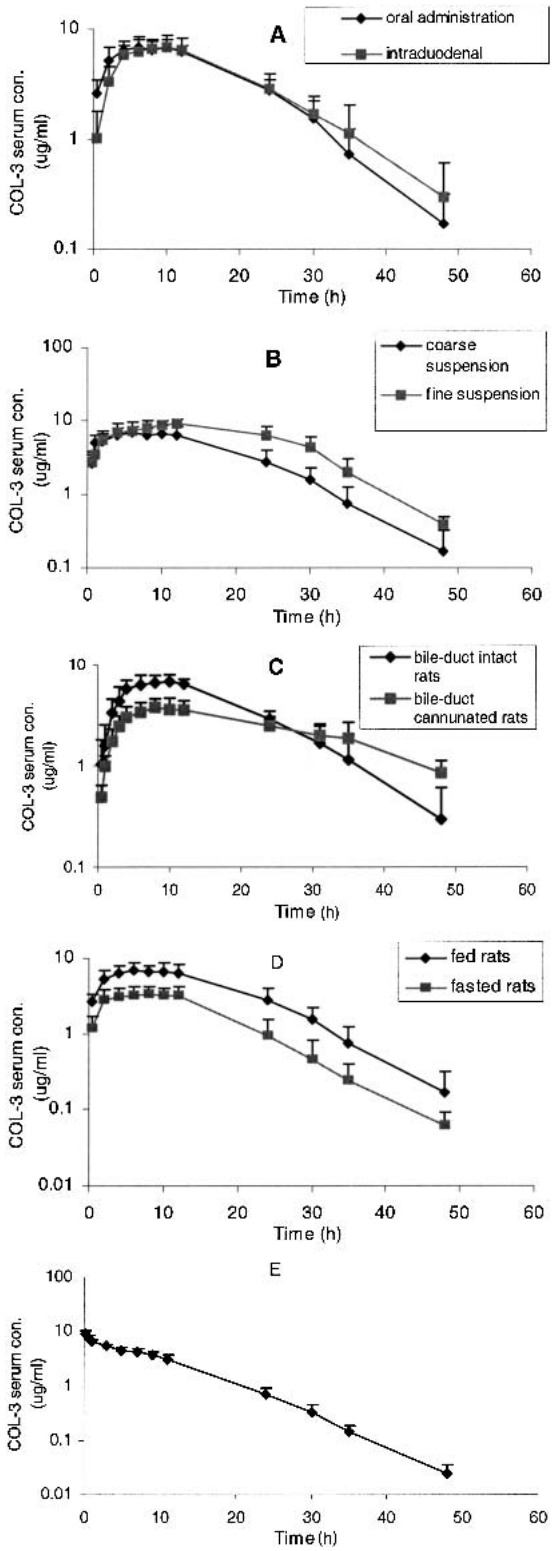

Fig. 2. The mean serum concentration-time profiles of COL-3 following (A) oral and intraduodenal administration of the coarse suspension to the fed rats; (B) oral administration of the fine and coarse suspensions to the fed rats; (C) intraduodenal administration of the coarse suspension to the bile-duct intact and cannulated fed rats; (D) oral administration of the coarse suspension to the fed and fasted rats; and (E) intravenous administration of the solution to the fasted rats. COL-3 was given at the extravascular dose of 30 mg/kg or at the intravenous dose of 10 mg/kg. Each data represent the mean (+ SD) of 6–9 rats

2B). The extent of absorption of COL-3 for the former (F = 100%) was about 1.6-fold of that for the latter (F = 62%). Reduction in particle size is known to be able to enhance the oral availability of many hydrophobic drugs (23), such as digoxin (24), griseofulvin (25), and panadiplon (26). For such poorly water-soluble drugs with absorption rate-limited by dissolution, reduction in particle size increases dissolution rate; thus more absorbable form (drug in solution) is available for absorption during the period of GI transit time.

The absence of endogenous bile had some effect upon the serum profile of COL-3 in the BDC rats (Fig 2C), since there was a substantial decrease in the rate of absorption of the drug, as measured by an increase in $t_{max,1}$ (by 45%) and $t_{max,2}$ (by 117%) and a decrease in $C_{max,1}$ (by 43%) and $C_{max,2}$ (by 61%) when compared with those in the intact rats. In addition, there was a small decrease in the extent of absorption in the BDC rats (F = 55%) compared with the intact rats (F = 62%). It has been widely reported that bile salts, such as sodium deoxycholate and sodium cholate, markedly improve the solubility and dissolution rate of, and thus enhance the GI absorption of, poorly water-soluble drugs (27,28), such as phenytoin (29) and vitamin E (30). It has been suggested that one of the steps in the GI absorption of relatively water-insoluble drugs is the preliminary solubilization of the drugs by bile salts (27). Therefore, the endogenous bile is likely to increase the dissolution rate as well as the solubility of COL-3 in the intestinal fluid and thus to enhance the absorption of COL-3 from the small intestine.

Food had a marked effect upon the absorption profile of COL-3 from a suspension in the rats (Fig 2D). The extent of COL-3 availability in the fed rats was increased by 128% compared to the fasted rats (Table 1: Group A vs. E), though both groups had a similar rate of absorption. It has been demonstrated that food affects the gastrointestinal physiology, such as gastric emptying, intestinal motility, acid secretion, bile secretion, enzyme secretion, and blood flow, that may influence drug absorption particularly for poorly water-soluble drugs (31). The delayed gastric emptying in the presence of food would likely result in a delayed drug absorption, but the longer gastric residence time would allow more time for dispersion and dissolution of poorly water-soluble drugs. In addition, the secretion of bile salts and the larger volume of gastrointestinal fluids after a meal may also increase the dissolution rate of such poorly water-soluble drugs like COL-3, thereby increasing the extent of absorption. It has been widely reported that the absorption of phenytoin and celecoxib is enhanced by food due to an improved dissolution rate (21,32). The enhancement of absorption of COL-3 by food in the present study provides another evidence to support the dissolution rate-limited absorption of COL-3 when given as a suspension.

COL-3 is highly hydrophobic and its solubility is pH-dependent. With its pK_a values of 5.6 (pK_{a1}) and 8.4 (pK_{a2}), COL-3 is sparingly soluble at the pH range 1 to 5 but highly soluble at pH above 7 (33). The pH of gastric contents varies between 3.3 and 5 in the rat (34). Bile secretion, enhanced during the fed conditions, increases the pH of the gastric contents emptied into the duodenum (35). In the absence of endogenous bile, the pH of the upper intestinal fluid may approach 5, at which the dissolution of COL-3 is unfavorable. The pH of the small intestinal fluid in the normal rat increases distally and varies from 6.5 in the duodenum to 7.1 in the

ileum (34). The dissolution of COL-3 would proceed faster at the lower than at the upper small intestine. In fact, the second serum concentration peak appeared to occur at approximately 10–12 h after a single oral dose administration. It is worthwhile to note that the dose was mostly given at around 9 AM, the time of the second peak was 7–9 PM, when food consumption begins with the dark cycle. The rat typically consumes food only during the dark cycle even though food is available *ad libitum*. Therefore, the second peak seen was due, at least in part, to increased absorption at the lower GI tract by increased bile excretion during food consumption.

The disposition of COL-3 after intravenous bolus injection was characterized by an elimination half-life of 5.81 h and a relatively large apparent volume of distribution of 1.10 l/kg in the rats (Group F). The terminal half-life value of COL-3 seen among the groups A, B, C, and E was similar to that after intravenous administration, suggesting that disposition rate limits elimination of COL-3 following its extravascular administration of the suspension to the normal (bile-duct intact) rats. In contrast, the terminal half-life value of COL-3 for the group D was prolonged by 174% to 15.9 h (Table 1) compared with that after intravenous administration, suggesting that absorption rate limits elimination of COL-3 following its extravascular administration of the suspension to the BDC rats with the bile flow diverged out of the body. The disappearance of the double peaks in the overall mean serum concentration-time curve for the BDC rats may simplify the estimation of the initial rate constant using the curve stripping (feathering) method. The initial half-life value of COL-3 estimated was 4.4 h, which appeared to lie within the range (4.4–6.7 h) of the elimination half-life value of the drug seen, further supporting that there was a flip-flop phenomenon in the BDC rats that the terminal phase of the serum concentration-time curve reflects absorption, whereas the initial portion of the curve reflects elimination, of COL-3. Thus, caution is needed when interpreting the terminal half-life of COL-3 after oral administration, especially in patients with a pathologic condition at which absorption or dissolution is unfavorable. It has been shown that the frequency of absorption restrictions (disorders) is increased with spreading (staging) of the carcinoma in cancer patients (36). Therefore, the clinically observed long and variable terminal half-life for COL-3 (ranging from 23.7–144.4 h) (7) could be at least in part, if not all, explained by a variable and slow dissolution rate-limited absorption of COL-3 in those cancer patients after oral administration of its solid dosage form (hard gelatin capsule).

In conclusion, the oral absorption of COL-3 from a suspension was dissolution rate-limited, which was supported by the evidence that the reduction in particle size and the presence of food and endogenous bile, could enhance the absorption of COL-3. The variability of bile concentration, food contents, and other physiologic factors affecting the dissolution of COL-3 in the gastrointestinal fluids may lead to a variable absorption rate of COL-3 along the gastrointestinal tract, which results in the double- or plateau-peak concentration in the COL-3 serum profile after oral administration.

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REFERENCES

1. H. M. Lee, L. M. Golub, and C. Cao. CMT-3, a non-antimicrobial tetracycline (TC), inhibits MT1-MMP activity: relevance to cancer. *J. Dent. Res.* **77**(IADR Abs.):926 (1998).
2. B. L. Lokeshwar. MMP inhibition in prostate cancer. *Ann. N.Y. Acad. Sci.* **878**:271–289 (1999).
3. L. A. Liotta and W. G. Stetler-Stevenson. Metalloproteinase and cancer invasion. *Sem. Cancer. Biol.* **1**:99–106 (1990).
4. W. G Stetler-Stevenson. S. Aznavoorian, and L. A. Liotta. Tumor cell interactions with the extracellular-matrix during invasion and metastasis. *Ann. Rev. Cell. Biol.* **9**:541–573 (1993).
5. M. G. Selzer, B. Zhu, N. L. Block, and B. L. Lokeshwar. CMT-3, a chemically modified tetracycline, inhibits bony metastases and delays the development of paralegia in a rat model of prostate cancer. *Ann. NY Acad. Sci.* **878**:678–682 (1999).
6. R. E. Seftor, E. A. Seftor, and J. E. Delarco. Chemically modified tetracyclines inhibit human melanoma cell invasion and metastasis. *Clin. Exp. Metastasis.* **16**:217–225 (1998).
7. M. A. Rudek, W. D. Figg, and V. Dyer. W Dahut, M, L. Turner, S. M. Steinberg, D. J. Liewehr, D. R. Kohler, J. M. Pluda, and E. Reed. Phase I clinical trial of oral COL-3, a matrix metalloproteinase inhibitor, in patients with refractory metastatic cancer. *J. Clin. Oncol.* **19**:584–592 (2001).
8. J. T. Bettany and R. G. Wolowacz. Tetracycline derivatives induce apoptosis selectively in cultured monocytes and macrophages but not in mesenchymal cells. *Adv. Dent. Res.* **12**:136–143 (1998).
9. S. Pinsuwan and F. A. Alvarez-Nunez. E. S Tabibi, and S. H. Yalkowsky. Degradation kinetics of 4-dedimethylamino sancycline, a new anti-tumor agent, in aqueous solutions. *Int. J. Pharm.* **181**:31–40 (1999).
10. R. L. Oberle and G. L. Amidon. The influence of variable gastric emptying and intestinal transit rates on the plasma level curve of cimetidine; an explanation for the double peak phenomenon. *J. Pharmacokinet. Biopharm.* **15**:529–544 (1987).
11. E. Lipka, I. D. Lee, P. Langguth, H. Spahn-Langguth, E. Mutschler, and G. L. Amidon. Celiprolol double-peak occurrence and gastric motility: nonlinear mixed effects modeling of bioavailability data obtained in dogs. *J. Pharmacokinet. Biopharm.* **23**: 267–286 (1995).
12. T. Ichikdawa, S. Ishida, Y. Sakiya, Y. Sawada, and M. Hanana. Biliary excretion and enterohepatic cycling of glycyrrhizin in rats. *J. Pharm. Sci.* **75**:672–675 (1986).
13. J. Hasselstrom and J. Sawe. Morphine pharmacokinetics and metabolism in humans: enterohepatic cycling and relative contribution of metabolites to active opioid concentrations. *Clin. Pharmacokinet.* **24**:344–354 (1986).
14. A. B. Suttle and K. L. B. Brouwer. Regional gastrointestinal absorption of ranitidine in the rat. *Pharm. Res.* **12**:1311–1315 (1995).
15. A. B. Suttle, G. M. Pollack, and K. L. B. Brouwer. Use of a pharmacokinetic model incorporating discontinuous gastrointestinal absorption to examine the occurrence of double peaks in oral concentration-time profiles. *Pharm. Res.* **9**:350–356 (1992).
16. Y. M. C. Wang and R. H. Reuning. A comparison of two surgical techniques for preparation of rats with chronic bile duct cannulae for the investigation of enterohepatic curculation. *Lab. Animal Sci.* **44**:479–485 (1994).
17. M. A. Rudek, C. L. March, K. S. Bauer Jr., J. M. Pluda, and W. D. Figg. High-performance liquid chromatography with mass spectrometry detection for quantitating COL-3, a chemically modified tetracycline, in human plasma. *J. Pharmaceu. Biomed. Anal.* **22**:1003–1014 (2000).
18. V. Mummaneni, G. L. Amidon, and J. B. Dressman. Gastric pH influences the appearance of double peaks in the plasma concentration-time profiles of cimetidine after oral administration in dogs. *Pharm. Res.* **12**:780–786 (1995).

19. S. L. Eeckhoudt, P. A. Evrard, and R. K. Verbeeck. Biliary excretion and enterohepatic cycling of r- and s-flurbiprofen in the rats. *Drug Metab. Dispos.* **25**:428–430 (1997).
20. R. L. Smith. Factors affecting biliary excretion. In R. L. Smith (eds.), *The Excretory Function of Bile*, Chapman and Hall, London, 1973, pp 16–34.
21. S. K. Paulson, M. B. Vaughn, S. M. Jessen, Y. Lawal, C. J. Gresk, B. Yan, T. J. Maziasz, C. S. Cook, and A. Karim. Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. *J. Pharmacol. Exp. Thr.* **297**:638–645 (2001).
22. M. Mayersohn. Principles of drug absorption. In G. S. Banker and C. T. Rhodes (eds.) *Modern Pharmaceutics*, 2nd ed., Marcel Dekker, New York, 1990, pp 23–90.
23. M. Gibaldi. Gastrointestinal absorption-physicochemical considerations. In M. Gibaldi (eds.) *Biopharmaceutics and Clinical Pharmacokinetics*, Lea & Febiger, Philadelphia, Pennsylvania, 1984, pp 49–57.
24. A. J. Jounela, P. J. Pentikainen, and A. Sothmann. Effect of particle size on the bioavailability of digoxin. *Eur. J. Clin. Pharmacol.* **8**:365–370 (1995).
25. W. L. Chiou and S. Riegelman. Absorption characteristics of solid dispersed and micronized griseofulvin in man. *J. Pharm. Sci.* **60**:1376–1380 (1971).
26. T. Nishihata, M. Ishizakd, and S. Yokohama. Effects of particle size of bulk drug and food on the bioavailability of U-78875. *Drug Dev. Ind. Pharm.* **19**:2679–2698 (1993).
27. T. R. Bates, M. Gibaldi, and J. L. Kanig. Rate of dissolution of griseofulvin and hexoestrol in bile salt solutions. *Nature* **210**: 1331–1333 (1966).
28. K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and M. Murakami. Effect of bile salts on the gastrointestinal absorption of drugs. *Chem. Pharm. Bull.* **18**:275–280 (1970).
29. D. Shinkuma, T. Hamaguchi, Y. Yamanaka, N. Mizuno, and N. Yata. Influence of bile on the gastrointestinal absorption of phenytoin in rats. *Chem. Pharm. Bull.* **33**:5023–5027 (1985).
30. M. G. Traber, H. J. Kayden, and J. B. Green. Absorption of water-miscible forms of vitamin E in a patient with cholestasis and in thoracic duct-cannulated rats. *Am. J. Clin. Nutr.* **44**:914–923 (1986).
31. A. Karim. Importance of food effect studies in early drug development. In K. K. Midha and T. Nagai (eds.), *Bioavailability, bioequivalence and pharmacokinetic studies*, Business Center for Academic Societies, Japan, Tokyo, Japan, 1996, pp 221–229.
32. C. Tschanz, W. W. Stargel, and J. A. Thomas. Interactions between drugs and nutrients. *Adv. Pharmacol.* **35**:1–26 (1996).
33. S. Pinsuwan, F. A. Alvarez-Nunez, S. E. Tabibi, and S. H. Yalkowsky. Spectrophotometric determination of acidity constants of 4-dedimethylamino sancycline (COL-3), a new antitumor drug. *J. Pharm. Sci* **88**:535–537 (1999).
34. T. T. Kararli. Gastrointestianl absorption of drugs. *Crit. Rev. Ther. Drug Carrier Syst.* **6**:39–85 (1989).
35. K. Takeuchi, O. Furukdawa, H. Tanake, and S. Okabe. Determination of acid-neutralizing capacity in rat duodenum. Influences of 16, 16-demethyl prostaglandin E2 and nonsteroidal antiinflamamatory drug. *Dig. Dis. Sci.* **31**:631–637 (1986).
36. R. Weiner, W. Hartig, R. Haupt, and M. Gierth. Small intestine absorption in cancer patients-Basis for enteral feeding therapy in oncology. *Z. Ernahrungswiss* **23**:157–170 (1984).