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pH-DEPENDENT UPTAKE OF IRINOTECAN AND ITS ACTIVE METABOLITE, SN-38, BY INTESTINAL CELLS

Kunihiko KOBAYASHI[1,2], Bernard BOUSCAREL[1,3]*, Yasushi MATSUZAKI[4], Susan CERYAK[1], Shoji KUDOH[2] and Hans FROMM[1]

[1]*Division of Gastroenterology and Nutrition, The George Washington University, Washington, DC, USA*
[2]*Department of 4th Internal Medicine, Nippon Medical School, Tokyo, Japan*
[3]*Department of Biochemistry and Molecular Biology, The George Washington University, Washington, DC, USA*
[4]*Division of Gastroenterology, Tsukuba University, Ibaraki, Japan*

Irinotecan (CPT-11) and its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), are believed to be reabsorbed by intestinal cells and to enter the entero-hepatic circulation, but there is little information to date. Our objective was to investigate the intestinal transport of CPT-11 and SN-38 in correlation with their associated cytotoxicity. Using either isolated hamster intestinal epithelial cells or/and human colon carcinoma HT29 cells, the uptake rates of [^{14}C]CPT-11 and [^{14}C]SN-38, both as respective non-ionic lactone form at acidic pH and anionic carboxylate form at basic pH, were investigated by the rapid vacuum filtration technique. The effect of physiologic intestinal luminal pH (6.2–8.0) on the uptake rate and cytotoxicity of SN-38 were estimated by the above method and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, respectively. The lactone forms of CPT-11 and SN-38 were transported passively, while the respective carboxylate form was absorbed actively. Uptake rates of both lactones were significantly higher than those of their carboxylates. Under physiologic pH, the respective uptake rates of CPT-11 and SN-38 were pH sensitive and decreased significantly by around 65%, at pH greater than 6.8. Furthermore, with decreasing pH, a higher uptake rate of SN-38 into HT29 cells correlates with a greater cytotoxic effect ($r = 0.987$). CPT-11 and SN-38 have absorption characteristics of weakly basic drugs such as short-chain fatty acids, suggesting that alkalization of the intestinal lumen may be critical to reduce their reabsorption and associated side effects. *Int. J. Cancer* 83:491–496, 1999.
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Irinotecan hydrochloride (CPT-11), a DNA topoisomerase I inhibitor (Kawato *et al.,* 1991), is broadening its clinical impact since it has shown not only clinical responses for many malignancies, but also survival benefits for patients with colorectal cancer (Cunningham *et al.,* 1998). However, CPT-11 does present major toxicities of leukopenia and diarrhea, which are now recognized as dose-limiting toxicities of this drug (Fukuoka *et al.,* 1992).

It is considered that the major metabolic pathways of CPT-11 and its metabolites are as follows: CPT-11 is hydrolyzed by liver carboxylesterase to an active metabolite, 7-ethyl-10-hydroxy-camptothecin (SN-38) (Rivory *et al.,* 1996*a*). A portion of SN-38 undergoes subsequent conjugation by the hepatic enzyme, UDP-glucuronyltransferase, to SN-38 β-glucuronide (SN38-Glu) (Atsumi *et al.,* 1991). Hepatic cytochrome P-450 3A enzymes metabolize CPT-11 to 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin, which has 500-fold weaker antitumor activity than SN-38 (Rivory *et al.,* 1996*b;* Haaz *et al.,* 1997). CPT-11, SN-38 and SN38-Glu have an α-hydroxy-3-lactone ring, which undergoes reversible hydrolysis at a rate that is mainly pH dependent (Fassberg and Stella, 1992). At physiologic pH and higher, the lactone form is unstable and the equilibrium favors hydrolysis to open the lactone ring and yield the carboxylate form. Under acidic conditions, lactone-carboxylate interconversion is shifted toward the lactone form. CPT-11, SN-38 and SN38-Glu are excreted into bile and along with it are released into the small intestinal lumen (Atsumi *et al.,* 1991; Lokiec *et al.,* 1995; Chu *et al.,* 1997*a,b*). Furthermore, although minor (Atsumi *et al.,* 1995), an additional pathway involves direct transport of CPT-11 and its metabolites from serum to lumen across the intestinal epithelial cells. Once in the intestine, SN38-Glu can be deconjugated in the cecum and colon to SN-38 by bacterial β-glucuronidase (Takatsuna *et al.,* 1996). CPT-11, SN-38 and SN38-Glu are believed to be reabsorbed to a certain extent by intestinal cells and to enter the entero-hepatic circulation.

However, to date, there is little information about the intestinal uptake and transport mechanism of CPT-11 and its derivatives. This knowledge is a critical step in the understanding of the mechanism by which CPT-11 induces diarrhea. In the present study, the uptake of CPT-11 and SN-38 by intestinal epithelial cells was estimated and correlated with their respective effect on cell toxicity.

MATERIAL AND METHODS

Drugs and chemicals

Both [^{14}C]SN-38 (3.68 MBq/mg) and [^{14}C]CPT-11 (1.47 MBq/mg) were kindly donated by Daiichi (Tokyo, Japan). Non-labeled CPT-11 and SN38-Glu were supplied by Yakult Honsha (Tokyo, Japan). [^{14}C]SN-38 was dissolved in DMSO (2% w/v final concentration) due to its hydrophobic property and poor solubility in water. At this final concentration, DMSO was confirmed to have no effect on the initial uptake of [^{14}C]CPT-11. The other compounds were dissolved in distilled water. [^{14}C]CPT-11 and [^{14}C]SN-38 were incubated overnight and at room temperature in 50 mM PBS, pH 3.0 or 9.0, to obtain the lactone and carboxylate form, respectively (Chu *et al.,* 1997*a,b*).

Preparation of cells

Intestinal cells were isolated from golden Syrian hamsters (100–130 g body weight), as previously described (Gore and Hoinard, 1993). All animals received humane care in compliance with the George Washington University guidelines. Following anesthesia by i.p. injection of sodium pentobarbital (Nembutal 100 mg/kg body weight), the hamsters were euthanized. The entire intestine was removed and the lumen was washed with 37°C Hanks' solution. Intestinal sacs of the ileum (12.5 cm from cecum) and jejunum (remaining small intestine) were rinsed with oxygenated buffer solution containing sodium citrate (96 mM NaCl, 1.5 mM KCl, 5.6 mM KH_2PO_4, 27 mM sodium citrate, pH 7.3) and incubated for 10 min in the same buffer at 37°C. The sacs were then emptied, filled with oxygenated buffer solution containing EDTA (140 mM NaCl, 16 mM Na_2HPO_4, 2 mM EDTA, 0.5 mM dithiothreitol, pH 7.3) and incubated for 8 min at 37°C. The buffer, containing intestinal cells, was recovered in 50 mL of Hanks' medium containing 0.5% BSA, pH 7.4, washed twice and adjusted to 10^6 cells/mL.

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*Correspondence to: Division of Gastroenterology and Nutrition, The George Washington University Medical Center, 2300 I Street, NW, #523 Ross Hall, Washington, DC, 20037, USA. Fax: +1(202) 994-3435.
E-mail: dombeb@gwumc.edu

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HT29 (human colon carcinoma cells, ATCC, Rockville, MD) cells were maintained in minimum essential medium (MEM) containing 10% fetal bovine serum at pH 7.4. They were used for both the uptake study and cytotoxicity assay described below.

Determination of the cellular uptake of [^{14}C]CPT-11 and [^{14}C]SN-38

Uptake of [^{14}C]CPT-11 and [^{14}C]SN-38 was measured by a rapid vacuum filtration technique (Gore and Hoinard, 1993). The cells in the respective Hanks' medium and MEM were incubated for 15 min at 37°C under permanent shaking. Uptake was started by the addition of [^{14}C]CPT-11 or [^{14}C]SN-38 at pH 3.0 and 9.0, respectively. At various time intervals, 100-µL sample aliquots were diluted into 3 mL of medium at 4°C to stop the uptake. The stop solution, containing the cells, was filtered through a glass microfiber filter (G4, Fisher, Pittsburgh, PA) under vacuum (20 psi). The filters were washed once with 5 mL of 0.5% BSA-containing medium (4°C) and once with 20 mL of medium (4°C). The filters were placed in vials containing 4 mL of scintillation fluid (Ultra Gold; Packard, Meriden, CT) and the radioactivity was determined in a β scintillation counter (LS3801; Beckman, Palo Alto, CA).

In studies of the effect of the metabolic inhibitor, 2,4-dinitrophenol (1 mM), the initial uptake rate of CPT-11 and SN-38 was determined following addition of this agent to the cell suspension 3 min prior to that of either [^{14}C]CPT-11 (20 µM) or [^{14}C]SN-38 (2 µM). The effects of S-(2,4-dinitrophenyl)-glutathione (DNP-SG) and SN38-Glu on the initial uptake rate of CPT-11 and SN-38 were also studied. The respective agent at a concentration of 200 µM was added to the cell suspension 7 min prior to the addition of the [^{14}C]-labeled compound.

The effect of physiologic pH on the initial uptake rate of CPT-11 and SN-38 was investigated in both hamster intestinal cells and HT29 cells. [^{14}C]CPT-11 (20 µM) and [^{14}C]SN-38 (2 µM), stored in PBS overnight at pH 6.2, 6.8, 7.4 and 8.0, were added to the cells and the uptake was measured over time.

Cytotoxicity assay

Rapid colorimetric assay for mitochondrial dehydrogenase activity was modified and used for the estimation of cytotoxicity of SN-38 (Mosmann, 1983). Briefly, HT29 cells were seeded into a 12-well plate (Falcon-3043; Lincoln Park, NJ). After 48 hr, SN-38 (0.4 µM) at pH 6.2, 6.8, 7.4 and 8.0 was added. After 24-hr exposure, the cells were washed twice and subjected to a drug-free incubation for 24 hr. Then, the cells were incubated with 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) for 4 hr. The blue formazan crystals were solubilized by addition of 10% n-dodecylsulfate sodium salt (SDS) in 0.01N HCl and overnight incubation. The formation of the blue formazan compound was spectrophotometrically determined at 560 nm (Ultraspec 4050; LKB, Bromma, Sweden).

Statistical analysis

The initial rate of uptake of CPT-11 or SN-38 was derived from the linear regression analysis of the respective regression line obtained from the plot of the uptake as a function of time. The initial rates of uptake were plotted against the corresponding concentration of the agent. The data were fitted by least-squares non-linear regression analysis using the equation $V = (V_{max} \times S)/(K_m + S) + K_d \times S$, where V represents the initial rate of uptake, V_{max} is the maximum rate of uptake, K_m is the apparent Michaelis constant, K_d is the rate of diffusion and S is the respective concentration of CPT-11 and SN-38.

Comparisons between 2 groups were evaluated by the Mann-Whitney rank sum test. Statistical significance of differences among more than 2 groups was determined by Kruskal-Wallis one-way analysis of variance on ranks. Multiple comparisons *vs.* control group or for all the paired groups were further performed by Dunn's method. The correlation between the initial rate of uptake and the cytotoxicity of SN-38 was plotted by a simple least-squares regression method.

RESULTS

Respective uptake of CPT-11 and SN-38, lactone and carboxylate, by intestinal cells

The time courses of the uptake of 20 µM [^{14}C]CPT-11 and 2 µM [^{14}C]SN-38 in both lactone and carboxylate forms by isolated jejunal cells are shown in Figure 1. The extrapolation of the uptake value at time 0 yields a positive intercept, indicative of non-specific binding, such as adsorption to labeled agents on the cell surface. The respective uptake of the lactone and carboxylate forms of both CPT-11 and SN-38 was linear for up to 90 sec. Therefore, the initial uptake rate was determined by linear regression fit of the uptake over this initial period of time. Comparison of the uptake rate between the lactone and carboxylate forms of the respective agent clearly showed a more rapid uptake of both CPT-11 and SN-38

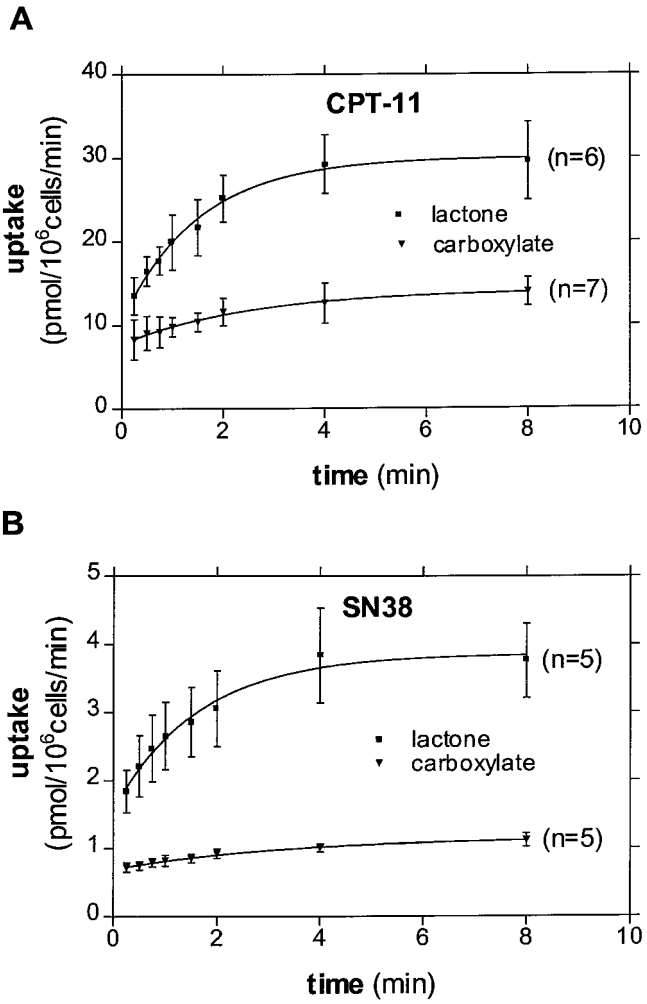

FIGURE 1 – Time-course of CPT-11 and SN-38 uptake by isolated intestinal cells. The uptake of *(a)* [^{14}C]CPT-11 (20 µM) and *(b)* [^{14}C]SN-38 (2 µM) in lactone and carboxylate form, respectively, by intestinal cells isolated from hamster jejunum was measured as a function of time. At time 0, the respective agent was added to the intestinal cell suspension maintained at 37°C under permanent shaking. At 15, 30, 45, 60, 90, 120, 240 and 480 sec, aliquots of cell suspension were removed and processed as described in Material and Methods. The results shown are mean ± SE.

TABLE I – INITIAL RATES OF UPTAKE OF CPT-11 AND SN-38 BY INTESTINAL CELLS[1]

	Jejunum		Ileum	
CPT-11				
Lactone	85.6 ± 8.6	$p < 0.001$	80.9 ± 10.6	$p = 0.01$
Carboxylate	31.1 ± 3.8		31.1 ± 4.2	
SN-38				
Lactone	6.76 ± 1.08	$p = 0.004$	6.14 ± 1.02	$p < 0.001$
Carboxylate	1.70 ± 0.27		1.51 ± 0.20	

[1]The initial rates of uptake of [^{14}C]CPT-11 (20 µM) and [^{14}C]SN-38 (2 µM), lactone and carboxylate, respectively, were compared. The results are expressed as pmol/10^6 cells/min and are the mean ± SE of 10 experiments. Mann-Whitney test was used for statistical analyses.

TABLE II – KINETIC PARAMETERS OF CPT-11 AND SN-38 UPTAKE BY INTESTINAL CELLS[1]

	Jejunum		K_d	Ileum		K_d
	K_m	V_{max}		K_m	V_{max}	
CPT-11						
Lactone	ND	ND	0.95 (0.15)	ND	ND	1.06 (0.28)
Carboxylate	51.3 (16.3)	146.9 (41.3)	<0.05 (<0.02)	50.5 (13.0)	157.3 (38.0)	<0.05 (<0.02)
SN-38						
Lactone[2]	ND	ND	2.38 (0.26)	ND	ND	1.87 (0.10)
Carboxylate[2]	ND	ND	0.44[3] (0.17)	ND	ND	0.42[3] (0.01)

[1]The data were fitted by least-square non-linear regression analysis using the equation $V = (V_{max} \times S)/(K_m + S) + K_d \times S$. V_{max} (pmol/10^6 cells/min) is the maximum rate of uptake, K_m (µM) is the apparent Michaelis constant, K_d (pmol/10^6 cells/min/µM) is the rate of diffusion and S (µM) is the concentration of either CPT-11 or SN-38. Values are mean and (SE). The major component of the uptake of CPT-11 lactone, SN-38 lactone and SN-38 carboxylate, respectively, was non-saturable and therefore, the K_m and V_{max} values were not determined (ND).–[2]Because of limited solubility, only concentrations of SN-38 up to 2 µM were investigated.–[3]Because SN-38 carboxylate is judged to be actively transported from the estimation of its uptake in the presence of dinitrophenol (Table III), these values are not considered to be physiologically relevant.

TABLE III – EFFECT OF DINITROPHENOL, SN38-Glu AND DNP-SG ON INITIAL UPTAKE RATE OF CPT-11 AND SN-38

	CPT-11 carboxylate		SN-38 carboxylate		CPT-11 lactone		SN-38 lactone	
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
Dinitrophenol (1 mM)[1]								
Mean	22.6	29.2	25.5	30.8	94.1	105.5	96.1	134.9
(SE)	(13.5)	(9.2)	(12.4)	(13.1)	(18.4)	(15.6)	(14.7)	(19.0)
p value[3] (n = 5)	0.016	0.008	0.008	0.016	NS[2]	NS	NS	NS
SN38-Glu (200 µM)								
Mean	108.9	93.9	40.1[5]	28.9[5]	88.9	NE	54.3	NE
(SE)	(22.1)	(14.3)	(11.1)	(11.2)	(15.3)		(20.6)	
DNP-SG (200 µM)								
Mean	103.2	105.8	32.0[5]	28.5[5]	105.4	NE	78.8	NE
(SE)	(17.0)	(36.3)	(9.9)	(11.9)	(12.7)		(24.4)	
p value[4] (n = 5)	NS	NS	0.007	0.020	NS		NS	

[1]Dinitrophenol, SN-38 glucuronide (SN38-Glu) or 2,4-dinitrophenyl-S-glutathione (DNP-SG) was added to the indicated cell suspension before the addition of [^{14}C]CPT-11 (20 µM) and [^{14}C]SN-38 (2 µM), respectively (for details, see Material and Methods). The initial uptake rate of CPT-11 and SN-38 in the presence of each compound was expressed as percentage (%) of control.–[2]Abbreviations. NE: not estimated; NS: not significantly different from control.–Differences between dinitrophenol and its control were evaluated by Mann-Whitney[3] test.–Differences among SN-38Glu, DNP-SG and their control were evaluated by Kruskal-Wallis[4] test.–The significant difference from respective control was analyzed according to Dunn's method ($p < 0.05$)[5].

lactones, as compared with their carboxylate form (Fig. 1). The intracellular concentration of CPT-11 and SN-38 is considered to be equal to the difference between the equilibrium concentration (plateau) and that at time 0 (y intercept). Thus, the intracellular concentration of the respective lactone at 8 min after starting the uptake study was also significantly higher than that of the respective carboxylate (Fig. 1). Similar figures were obtained when isolated cells from ileum were used (data not shown).

Table I summarizes the respective initial uptake rate of 20 µM [^{14}C]CPT-11 and 2 µM [^{14}C]SN-38 by jejunal and ileal cells. CPT-11 and SN-38 lactone were significantly more rapidly taken up than their carboxylate forms in cells from both intestinal regions

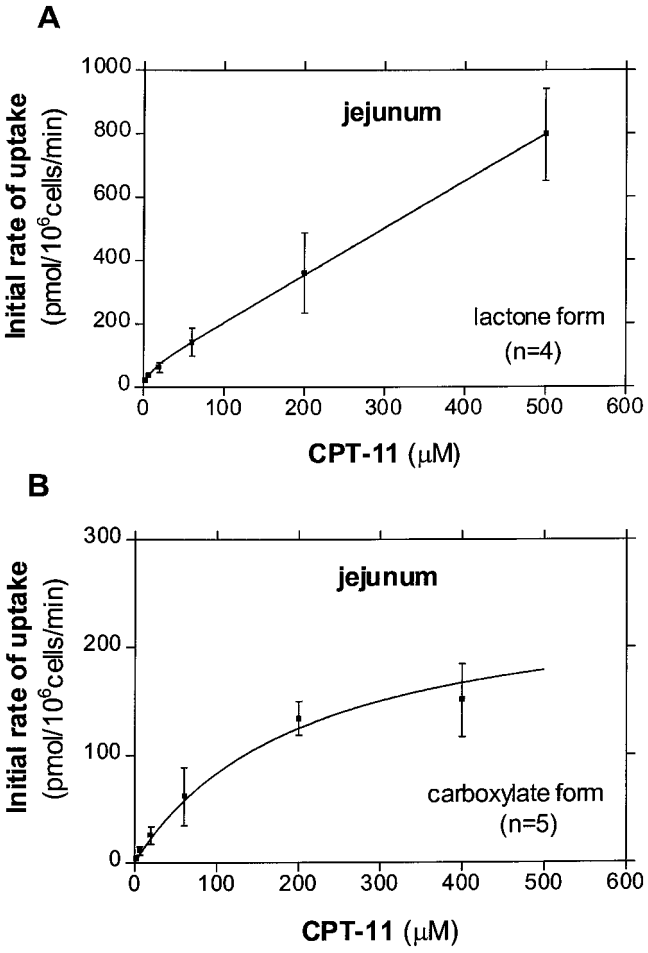

FIGURE 2 – Relationship between initial rate of uptake of CPT-11 and its concentration. The initial rate uptake of [^{14}C]CPT-11 in *(a)* lactone and *(b)* carboxylate forms by jejunal cells was determined from the linear slope of the cellular uptake over the initial 90-sec incubation period. The data were fitted by least-square non-linear regression analysis using the equation $V = (V_{max} \times S)/(K_m + S) + K_d \times S$. Similar figures were obtained when isolated cells from the ileum were used (data not shown).

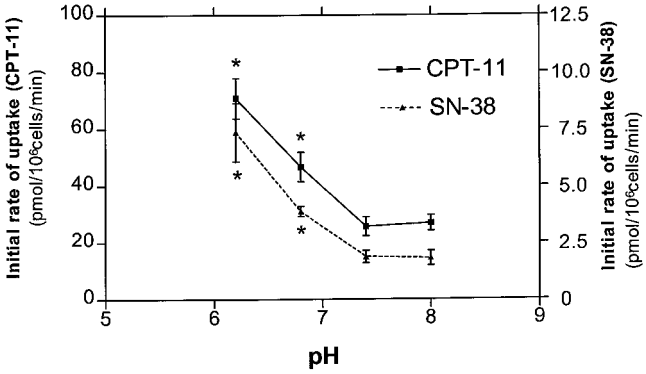

FIGURE 3 – Effect of pH on the initial uptake rate of isolated intestinal cells. *(a)* [^{14}C]CPT-11 (20 μM) and *(b)* [^{14}C]SN-38 (2 μM) were dissolved in PBS at pH 6.2, 6.8, 7.4 and 8.0 overnight. The uptake study was initiated by adding the compounds to Hanks' solution containing isolated intestinal cells. The comparative initial rate of uptake as a function of pH was analyzed by Kruskal-Wallis test (CPT-11, $p < 0.001$; SN-38, $p < 0.001$) and Dunn's method ($^*p < 0.05$).

but without significant differences between jejunal and ileal cells, using the Mann-Whitney test.

Transport system of CPT-11 and SN-38 lactone and carboxylate

The respective initial uptake rate of CPT-11 lactone and carboxylate was plotted as a function of the concentration, and the data were fitted by least-squares non-linear regression analysis using the equation $V = (V_{max} \times S)/(K_m + S) + K_d \times S$ (Table II, Fig. 2). In both jejunal and ileal cells, the predominant component of the uptake of CPT-11 lactone was non-saturable, suggesting uptake by either passive diffusion or fluid-phase endocytosis. For CPT-11 carboxylate, the saturable component of the curve was characterized by a maximum rate of uptake (V_{max}) of 147 and 157 pmol/10^6 cell/min and a Michaelis constant (K_m) of 51.3 and 50.5 μM in jejunal and ileal cells, respectively. The minor non-saturable component was characterized by a diffusion constant (K_d) < 0.05 pmol/10^6 cell/min/μM and represented less than one twentieth of that for CPT-11 lactone in cells of both intestinal regions (Table II). This indicated that CPT-11 carboxylate uptake involved mainly an active transport.

The maximum concentration of SN-38 used in this study was lower than 2 μM due to the poor solubility of the compound. In this range of concentrations, the uptake of SN-38 lactone and carboxylate was mostly non-saturable. However, considering that the saturation was found in CPT-11 carboxylate uptake at the concentration of more than 60 μM, the determination of the saturable and unsaturable component of the uptake of SN-38 was considered to be unfeasible.

Metabolic poisons, such as 2,4-dinitrophenol, which interferes with cell metabolism and reduces energy-producing reactions, are known inhibitors of carrier-mediated transport mechanism (McCloud *et al.*, 1994). Therefore, 2,4-dinitrophenol was used in our study to determine the respective mechanism of uptake of CPT-11 and SN-38 in both lactone and carboxylate forms. The results of this study are summarized in Table III. Although the uptake rates of both CPT-11 and SN-38 lactones were not significantly affected, those of CPT-11 and SN-38 carboxylates were significantly reduced by over 70% in the presence of 2,4-dinitrophenol, indicating that both CPT-11 and SN-38 in the carboxylate forms were actively transported.

DNP-SG is an organic anion. SN38-Glu has not only an α-hydroxy-3-lactone ring, but also a carboxyl group in its glucuronide. It was reported that both SN38-Glu lactone and SN-38-Glu carboxylate were secreted into bile by the hepatic canalicular multispecific organic anion transporter (Chu *et al.*, 1997*a*,*b*), suggesting that both forms have a negative-ion charge. Table III shows the respective competitive effect of DNP-SG and SN38-Glu on the initial rate of uptake of CPT-11 and SN-38. DNP-SG and SN38-Glu significantly inhibited the uptake of the carboxylate form of SN-38 by over 60% while that of CPT-11 carboxylate remained unchanged. The uptake rates of the lactone forms of CPT-11 and SN-38 were not significantly affected by the presence of either DNP-SG or SN38-Glu.

Effect of pH on the intestinal uptake of CPT-11 and SN-38

The interconversion between the lactone and carboxylate forms of CPT-11 and SN-38, respectively, is reversible and pH-driven (Fassberg and Stella, 1992). The luminal pH in the jejunum and ileum was reported to range from 6.0 to 7.0 and from 6.5 to 8.0, respectively (Charman *et al.*, 1997). The effect of physiologic pH (6.2–8.0) in the intestinal lumen on the initial uptake rate of 20 μM [^{14}C]CPT-11 and 2 μM [^{14}C]SN-38 was studied using hamster intestinal and HT29 cells. The results with hamster intestinal cells summarized in Figure 3 show that the uptake rate of CPT-11 and SN-38 was significantly decreased by around 65% at a pH greater than 6.8.

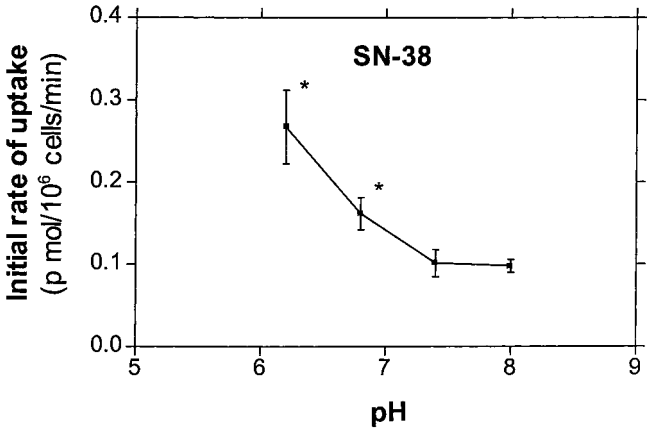

FIGURE 4 – Effect of pH on the initial uptake rate of HT29 cells. [^{14}C]SN-38 (2 µM) were dissolved in PBS at pH 6.2, 6.8, 7.4 and 8.0 overnight. The uptake study was initiated by adding the compounds to Hanks' solution containing HT29 cells. The comparative initial rate of uptake as function of pH was analyzed by Kruskal-Wallis test ($p < 0.001$) and Dunn's method ($^*p < 0.05$).

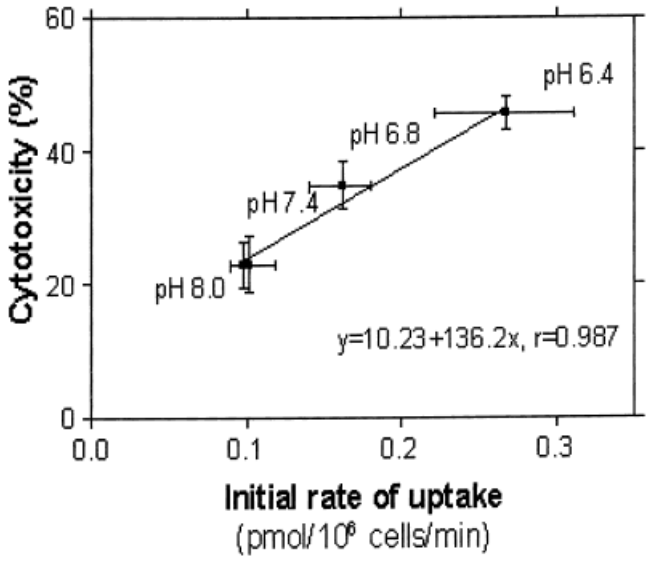

FIGURE 5 – Relationship between the initial uptake rate and the cytotoxicity of SN-38. Using HT29 cells, the effect of physiologic pH on the initial uptake rate of 2 µM [^{14}C]SN-38 was estimated as described in the legend of Figure 3. The 0.4 µM SN-38-induced cytotoxicity in HT29 cells was studied by the described MTT assay. The relationship between the initial rate of uptake and the cytotoxicity of SN-38 was plotted by a simple least-squares regression method.

Relationship between the initial uptake rate and the cytotoxicity of SN-38

It has been reported that CPT-11 administration induced apoptosis in intestinal epithelium and disrupted it *in vivo* (Ikuno *et al.,* 1995). However, isolated hamster intestinal cells are not good models to estimate the cytotoxic effect of SN-38 due to their limited viability to around 2 hr (Gore and Hoinard, 1993). Therefore, HT29 cells were used to study the comparative effects of physiologic pH on both the initial uptake rate of 2 µM [^{14}C]SN-38 and the cytotoxicity of 0.4 µM SN-38. The initial rate of uptake of SN-38 was lower in HT29 cells than in isolated hamster intestinal cells (Figs. 3, 4). However, as observed in isolated hamster intestinal cells, the uptake rate of SN-38 in HT29 cells was significantly greater at pH 6.2 and 6.8 than at pH 7.4 and 8.0 (Kruskal-Wallis test: $p = 0.008$, Dunn's method: $p < 0.05$) (Fig. 4). The cytotoxicity of SN-38 for HT29 cells was significantly higher at pH 6.2 and 6.8 than at pH 7.4 and 8.0 (Kruskal-Wallis test: $p = 0.007$; Dunn's method: $p < 0.05$). Figure 5 shows the relationship between the initial rate of uptake of [^{14}C]SN-38 and the cytotoxicity of SN-38, indicating that, with decreasing pH, a higher uptake rate correlated with a more cytotoxic effect.

DISCUSSION

Using hamster intestinal cells, we have studied the transport mechanism of CPT-11 and SN-38. The non-ionic lactone forms of both CPT-11 and SN-38 were absorbed mainly through a passive mechanism. There were no differences either in the transport mechanism or in the initial uptake rates between jejunal and ileal cells (Tables I, III). Although not shown, similar results were also observed when the uptake of CPT-11 and SN-38 was performed using both cecal and colonic cells (Kobayashi *et al.,* 1998*a*). Furthermore, our experiments show that uptake rates of both lactones of CPT-11 and SN-38 were significantly higher than those of their carboxylates (Table I).

Our results clearly showed that CPT-11 and SN-38 in anionic carboxylate form were taken up by intestinal cells through active mechanisms. However, in contrast with CPT-11 carboxylate, the initial rate of intestinal uptake of SN-38 carboxylate was significantly inhibited by DNP-SG and SN-38-Glu (Table III). These results provide support for different intestinal transporters for CPT-11 and SN-38 carboxylate as suggested by Chu *et al.* (1997*a*,*b*) in the liver. They have indeed previously reported that the respective hepatic canalicular excretion of the anions of SN-38 carboxylate, SN-38-Glu lactone and carboxylate involved cMOAT and was different from that of the anion CPT-11 carboxylate.

Under the physiologic range of pH of the intestinal lumen reported by Charman *et al.* (1997), the initial uptake rate of CPT-11 and SN-38 was several times greater at acidic pH (6.2 and 6.8) than at neutral or alkaline pH (7.4 and 8.0) (Fig. 3). The pH dependency of the interconversion of lactone and carboxylate has been previously reported. Fassberg and Stella (1992) have reported that 11.7% and 90.8% of camptothecin was in its lactone form at pH 7.6 and 5.6, respectively. Furthermore, 10% and 90% of SN-38 and CPT-11 were in their lactone and carboxylate form at pH 7.4 and 6, respectively (Burke and Mi, 1994). Finally, the ratio of SN-38 lactone is 45% at pH 6, 11.1% at pH 7, and 5% at pH 7.4 (Akimoto *et al.,* 1994 and personal communication).

The results discussed above suggest that the mechanism of uptake of CPT-11 and SN-38 by intestinal cells closely resembles that of short-chain fatty acids and weakly basic drugs, of which absorption characteristics are: (1) at acidic pH, the non-ionic form is transported passively; (2) at neutral/basic pH, the anionic form is absorbed actively; (3) the uptake rate of the non-ionic form (lactone) is higher than that of the anionic form (carboxylate) (Charman *et al.,* 1997; Bugaut, 1987). In addition, Charman *et al.* (1997) reported the importance of pH in the intestinal lumen relative to the pKa of poorly water-soluble, weakly basic drugs, *i.e.,* lower pH in the intestinal lumen induces higher uptake of weakly basic drugs. Therefore, following this principle, alkalization of intestinal luminal content should reduce the intestinal uptake of CPT-11 and SN-38.

Furthermore, SN-38 exhibits strong cytotoxicity, SN38-Glu is a deactivated glucuronidated form of SN-38 and CPT-11 is much less cytotoxic compared to SN-38 (Kawato *et al.,* 1991). Accumulation of SN-38 in the intestine was shown in rats (Atsumi *et al.,* 1995) and was believed to be responsible for the diarrhea attributed to CPT-11 administration in nude mice (Araki *et al.,* 1993). Disruption of the intestinal epithelium in the cecum was observed in mice and rats with diarrhea after CPT-11 administration (Takatsuna *et al.,* 1996; Ikuno *et al.,* 1995; Araki *et al.,* 1993). The diarrhea induced by CPT-11 administration in humans was reported to be

secretory diarrhea (Bleiberg and Cvitkovic, 1996). However, as reported in animal models, we observed lethal small-intestinal injury associated with CPT-11-induced side effects in patients (Kobayashi *et al.,* 1998*b*). The autopsy revealed the presence of pseudomembranous jejuno-ileitis, the appearance of which under light microscopy was characterized by disruption of the intestinal epithelium, suggesting that damage diarrhea could occur in severe cases. These reports support a predominant role of SN-38 in both epithelial damage and diarrhea.

Therefore, the relationship between the cellular uptake of SN-38 and its associated cytotoxicity was also estimated in the present study. We found that the cellular uptake and cytotoxicity of SN-38 in HT29 cells was pH dependent and that the cytotoxicity correlated well with the initial uptake rate (Fig. 5). As previously described, it is considered that at acidic pH, the predominant form of SN-38 is lactone. This would lead to both a greater cellular uptake and intracellular concentration of SN-38 lactone. Since SN-38 is active mainly as the lactone form, while SN-38 carboxylate exhibits only minor topoisomerase I-inhibitory activity (Kawato *et al.,* 1991), this should be associated to increased cell death. Therefore, one possible mechanism for CPT-11-induced diarrhea might include the reabsorption of SN-38 lactone by the intestinal epithelium, resulting in structural and functional injuries to the intestinal tract.

In summary, we have estimated the uptake of CPT-11 and SN-38 by intestinal epithelial cells. CPT-11 and SN-38 lactone are both passively transported, while both CPT-11 and SN-38 carboxylate are actively absorbed. The uptake rate of CPT-11 and SN-38 lactone is several times greater than that of the respective carboxylate form. Furthermore, the higher uptake rate of SN-38 is associated with an increased cytotoxic effect on HT29 cells. Our findings suggest that the conversion to carboxylate would reduce the cellular uptake of both CPT-11 and SN-38. Consequently, our findings provide support for alkalization of the intestinal lumen as a possible mechanism to reduce reabsorption of CPT-11 and SN-38 in clinical practice. It is possible that limited intestinal reabsorption in turn modulates the bioavailability of this drug circulating entero-hepatically and reduces the toxic side effects of SN-38 on the intestinal epithelium.

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