Scientific
Research

Bicarbonate Attenuates Irinotecan-Induced Cytotoxicity through Regulation of Both Extracellular and Intracellular *pHs* in Intestine Cell Line

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ABSTRACT

The anti-cancer therapy of irinotecan (CPT-11) is often limited due to severe late-onset diarrhea. Because the higher toxic form of CPT-11/its active metabolite (SN-38) is produced at acidification, the usefulness of oral sodium bicarbonate treatment against the CPT-11/SN-38-induced intestinal injuries and diarrhea has been confirmed. However, the roles of bicarbonate have been suggested to affect not only intestinal *pH* environment but also intracellular *pH* and CPT-11/SN-38 dynamics. The present study proposed to clarify the hypothesis in CPT-11/SN-38-exposed colon cell line in various *pH* conditions adjusted by bicarbonate. HT29 cell pre-exposed to ~1.0 μM SN-38 lactone or carboxylate forms was incubated at different *pH* adjusted by either bicarbonate or HCl/NaOH. The degrees of SN-38-induced cell injury depended on the higher proportion of the toxic form (lactone) of SN-38 rather than mere *pH* condition of medium. Apoptosis and cell injury induced by SN-38 were significantly inhibited by bicarbonate in a dose-dependent manner. Intercellular *pH* acidification induced by SN-38 was significantly prevented by 30 mM bicarbonate. Cell cytotoxicity of SN-38 depended on not only extracellular but also intracellular *pH* that converts the SN-38 form, while the intracellular acidification was prevented by bicarbonate. The multiple regulations of bicarbonate on both exracellular and intracellular *pH* would be essential mechanism against intestinal cell injury by CPT-11/SN-38.

Keywords: Bicarbonate; SN-38; CPT-11; *pHi*; Anti-Cancer Drug; Intestine

1. Introduction

Irinotecan hydrochloride (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin) is a water-soluble derivative of camptothecin, an alkaloid isolated from Camptotheca acuminata, and presents a wide spectrum of antitumor activity through the inhibition of DNA topoisomerase I [1]. This chemotherapeutic agent has been approved by the FDA for treatment of colorectal cancer in the United States, but has shown clinical responses for many other malignancies such as lung, gastric, pancreatic, cervical, and ovarian cancers as well as leukemia and lymphoma [2-6]. However, this agent has major limitation in the therapeutic use for the

patients with cancer due to severe side effect as the lethal delayed diarrhea [7,8] including the marked intestinal injury and the presence of pseudomembranous jejuno-ileitis [9].

CPT-11 is hydrolyzed to active 7-ethyl-10-hydrocy-camptothecin (SN-38) by hepatic carboxylesterase [10], and SN-38 has at least a 100-fold more potent antitumor effect than CPT-11 [1]. The ability of both CPT-11 and SN-38 relies on *pH*. Both CPT-11 and SN-38 have a labile *α*-hydroxy-3-lactone ring, which undergoes reversible hydrolysis at a rate that is mainly *pH*-dependent [11]. At acidic condition, the lactone ring of CPT-11/SN-38 closes, and this form is called as a lactone form. On the other hand, the lactone ring opens at physiological *pH* and higher, and yields a carboxylate form. The carboxy-

late form is a less potent inhibition of the topoisomerase I and has much weaker antitumor activity than its lactone counterpart. Therefore, one of the possible strategies for prevention from the CPT-11-induced side effects might be manipulating the *pH*-dependent interconversion. Indeed, we and others have previously confirmed the oral bicarbonate (HCO_3^-) administration prior to and/or during CPT-11 treatment avoided acidifications in intestinal lumen and stool, and consequently, could decrease the incidence of side effects including delayed diarrhea, nausea, vomiting, myelotoxicity, and lymphocytopenia in the patients with lung cancer [12], advanced gastrointestinal cancer [13] or colorectal cancer [14], and the experimental animal model [12,15]. Therefore, it has been believed that oral HCO_3^- treatment is effectiveness on the attenuation of the side effects in CPT-11 anticancer therapy through the keeping of intestinal alkalization.

Additionally, our previous studies also confirmed that there was significant difference in the initial uptake rate of CPT-11/SN-38 by isolated hamster intestinal cells between the respective lactone and carboxyrate forms [9,15]. The uptake rate of the lactone form that is passively transported was higher than the carboxyrate form that is actively absorbed. Therefore, the regulation of ambient *pH* environment is also important factor for the intracellular uptake of CPT-11/SN-38.

Furthermore, intracellular *pH* (*pHi*) is also associated with anti-cancer action of the agent, because it generally influences the cellular proliferation and death [16-19]. Because intracellular acidification is an early event in apoptosis and also essential in genomic DNA destruction [17], it has been also considered as one of the multiplier factors to emphasize the inhibitive action of SN-38 on the topoisomerase I [20]. In addition to the regulation of ambient *pH*, HCO_3^- has been reported to act as a regulator of *pHi* in some cells through Na^+-dependent Cl^-/HCO_3^- exchange, Na^+-independent Cl^-/HCO_3^- exchange, and Na^+-HCO_3^- cotransport [21-23]. So, it is suggested that HCO_3^- might have another potential protective action against SN-38 through the regulation of the *pHi*.

While the oral HCO_3^- treatment has demonstrated scientific and clinical benefits on CPT-11 treated anticancer therapy, there is few understanding of its action. In the present study, we purposed to clarify the implication of HCO_3^- on *pH*-dependent CPT-11/SN-38 toxicity in the human colon adenocarcinoma HT29 cell line under various culture *pH* environments adjusted with/without HCO_3^-.

2. Materials and Methods

2.1. Materials

SN-38 was supplied by Yakult Honsha Co., Ltd. (Tokyo,

Japan) and was 98% - 99% pure as judged by gas-liquid chromatography. Nigericine was purchased from Sigma (St. Louis, MO). Snarf1-AM was from Calbiochem (San Diego, CA). Other chemicals were of the highest purity available. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco®, Invitrogen Corporation (Carlsbad, CA). For the culture conditions, the HT29 cell line (ATCC®, Manas- sas, VA) was maintained in DMEM supplemented with 10% heat-inactivated FBS, as previously described [9,24, 25]. The HT29 model was selected since we have previ- ously reported a similar respective mechanism of uptake of SN-38 lactone and carboxylate to that reported in isolated intestinal cells [26]. Moreover, HT29 cells allow for long-term studies that are not possible using the isolated enterocyte model.

2.2. Chemical (SN-38) Preparation

SN-38 stock solution was prepared by dissolution with DMSO due to highly hydrophobicity. The SN-38 stock solution was incubated with 50 mM PBS, *pH* 3.0 or 9.0, overnight at RT, as a 100-fold dilution, to obtain the lactone and carboxylate forms, respectively [27]. At this final concentration, DMSO was confirmed to have no effect on the initial uptake of the agent [26]. SN-38 at concentrations up to 5 μM did not significantly change the *pH* of the culture medium. The resultant solution was 98% lactone form at *pH* 3.0 and 97% carboxylate form at *pH* 9.0 (HPLC determination by Dr. K. Arimori and T. Hidaka, Dept. Pharmacy, Miyazaki Medical School, Miyazaki, Japan). For cell culture, SN-38 was diluted to final concentrations of 0.5 or 1.0 μM in DMEM.

2.3. Effect of Extracellular *pH* on SN-38-Induced Cytotoxicity

HT29 cells seeded on a 96-well plate as density at 1×10^4 cells/well were cultured for 24 h, and then exposed to either 0 or 0.5 μM SN-38 lactone/carboxylate in DMEM for 2 h that the exposed period does not affect the interconversion of SN-38 [10,28]. The cells were then washed twice with PBS, and further incubated in DMEM without the SN-38 at *pH* 6.2 or 7.2 set by HCl/NaOH for 96 h. Thereafter, cell viability was measured by the MTT assay, and expressed as a relative ratio to control (0 μM SN-38) in each culture condition.

2.4. The Effect of Bicarbonate-Adjusted *pH* before and after SN-38 Exposure

HT29 cells seeded on a 24-well plate at a density of 5×10^4 cells/well were cultured in standard condition for 24 h, and then, were incubated with DMEM containing 25 mM HEPES and increasing concentrations (5, 20, 44, 100 mM) of HCO_3^- for 2 h prior to SN-38 exposure.

The HCO_3^- concentrations resulted in the *pH* adjustment at 7.0, 7.2, 7.4, and 7.8, respectively. The osmolality was maintained constant among the different HCO_3^- buffers. After the incubation with HCO_3^-, cell was exposed to either 0.5 or 1.0 µM SN-38 lactone for an additional 2 h. Thereafter, cell was washed twice with PBS, and further incubated for an additional 96 h with the respective HCO_3^- containing culture medium without SN-38. In parallel experiments, HT29 cell was cultured without HCO_3^- prior to the SN-38 exposure, and were incubated with the different concentrations of HCO_3^- during the additional 96 h exposure.

2.5. Cell Viability in Various *pH* Conditions with/without Bicarbonate

HT29 cell cultured under standard condition containing DMEM with 44 mM HCO_3^-, at *pH* 7.4, was exposed to either 0 or 1.0 µM SN-38 lactone for 2 h. Thereafter, the washed cell was further incubated for an additional 48 h with 5-set points of *pH* at 6.8, 7.0, 7.4, 7.8, or 8.2 in culture medium adjusted by either increasing concentrations of HCO_3^- [5, 10, 20, 44, 100 mM, respectively; HCO_3^- (+)] or NaOH with 5 mM HCO_3^- [HCO_3^- (−)]. During cell culture, acidification of medium is usually induced due to respiration of cells. Therefore, in the present experiment, the cell was cultured with different media in each condition to maintain *pH* during culture. DMEM contained 2.5 mM·L-glutamate, 1 mM sodium pyruvate, and 10% FBS, and each culture medium was constituted as shown in **Table 1**. The constitution of the medium was based on a previous study by Eagle *et al.* [29] and further modified in the present study. Cell proliferation after culture in these conditions was determined by the MTT assay.

For detection of apoptotic cells in the various pH conditions either with or without HCO_3^-, following exposure to SN-38 lactone, the cell was incubated with 3-set points of *pH* at 6.8, 7.0, or 7.8 with increasing HCO_3^- concentrations [HCO_3^- (+)] or without HCO_3^- [HCO_3^- (−)] for an additional 48 h.

2.6. Cell Viability Assay

Cell viability was analyzed as cell proliferation and apoptosis by the MTT assay [30] and flow cytometry, respectively. In the MTT assay, following incubation of the cell with the indicated concentration of the agents, the medium was removed and the cell was further incubated with 0.5 mg/mL MTT in medium for 4 h at 37˚C. The blue formazan compound was solubilized with methanol containing 0.01 N HCl. The absorbance was determined at 560 nm, with 670 nm used as a reference in a spectrophotometer. In the flow cytometry, apoptotic HT29 cell was determined by annexin V and propidium iodide (PI) double staining using a commercially available kit (Annexin V-EGFP Apoptosis Detection Kit, BioVision, Inc., Mountain View, CA). Following the respective culture condition, the cell was harvested with trypsin and suspended with annexin V-enhanced green fluorescent protein (EGFP) and PI, and then analyzed by flow cytometry (FacSort, Becton Dickinson, San Jose, CA).

2.7. Intracellular *pH* Determination

HT29 cell plated at 1×10^6 cells per well was incubated with and without 0.5 µM SN-38 lactone for 2 h in culture medium containing increasing concentrations of HCO_3^- (10 - 58 mM). After removal of SN-38, the cell was maintained under the same culture conditions for an additional 4 h period before being loaded with 5 µM Snarf-1 acetoxymethyl acetate for 20 - 40 min at 37˚C. The cell was then washed, and the respective cellular fluorescence level was determined with the ACAS 570 laser scanner cytometer (Meridian Instruments Inc., Okemos, MI). A *pH* standard curve (6.8, 7.2, 7.4, 7.6, and 8.0) using high potassium concentration (100 mM) was determined for each series of experiments using either PIPES for buffer *pH* < 7 or HEPES for buffer *pH* > 7 and 10 µM nigericin. The cell membrane potassium ionophore nigericin causes the interior of the cells to equilibrate with the external *pH* of the buffer. The cells were excited with a 514 nm argon laser beam (5 W, Innova 90/5, Coherent) and the 587 nm and 640 nm emissions were simultaneously collected. The fluorescence intensity at each wavelength was detected using a photomultiplier tube. Images were acquired by scanning several fields of cells. Each image consisted of 3000 - 5000 discrete sample points. The ratio of the 640/587 nm emission data was used to determine the *pHi*. On

Table 1. Conditions of culture medium at different *pHs*.

HCO_3^- (+)	*pH* 6.8	*pH* 7.0	*pH* 7.4	*pH* 7.8	*pH* 8.2
NaHCO$_3$ (mM)	5	10	20	44	100
Hepes (mM)	25	25	25	25	25
HCO_3^- (−)	*pH* 6.8	*pH* 7.0	*pH* 7.4	*pH* 7.8	*pH* 8.2
NaHCO$_3$ (mM)	5	5	5	5	5
Trice (mM)	10	0	0	0	0
Hepes (mM)	25	25	20	20	20
Tris-base (mM)	0	0	25	25	25
NaH$_2$PO$_4$ (mM)	10	10	0	0	0

The cell was incubated in 5% and 0% CO$_2$, in HCO_3^- (+) and HCO_3^- (−), respectively.

average, the data were collected from 12 - 20 successive scans.

2.8. Statistical Analysis

Statistically significant differences were assessed by applying the Student's unpaired *t*-test, paired *t*-test, or analysis of variance (ANOVA) where applicable with an α level of 0.05. Control experiments, using only the vehicle for solubilizing the respective agent, were performed in parallel. The results were expressed as the mean ± SD of the mean based on the number of studies. Each experiment was performed in duplicate or triplicate.

3. Results

3.1. Effect of Extracellular *pH* on SN-38-Induced Cellular Toxicity

Table 2 shows the cell viability of HT29 cell after exposure to either the SN-38 lactone or carboxylate form in different two *pH* conditions at 7.4 or 6.2 adjusted by NaOH/HCl. At *pH* 7.4, cell viability was significantly decreased in the exposure to carboxylate form compared to that in the control, and was further and significantly decreased in the exposure to lactone form compared to that in other conditions. The significantly decreased cell viability in the lactone form rather than the carboxylate form was also observed at *pH* 6.2. In addition, the viability in lactone form at *pH* 6.2 was significantly lower than carboxylate form at *pH* 7.4. Compared between *pH* 7.4 and 6.2, the toxic level of SN-38 lactone was almost similar. These results are in agreement with the previous study reporting that cell cytotoxicity of SN-38 depended on the form, *i.e.*, lactate or carboxylate forms rather than extracellular *pH* [15].

3.2. The Difference of Bicarbonate Treatment between before and after SN-38 Exposure at Various *pH* Conditions in the Cellular Toxicity

Figure 1 presents the cell viability of HT29 cells cult-

Table 2. Effect of medium *pH* on SN-38 toxicity.

Agents	*pH* Culture medium	Cell viability (%)
Control	7.4	100
SN-38 Lactone	7.4	45.3 ± 1.1[*†]
SN-38 Carbonate	7.4	66.6 ± 3.8[*]
SN-38 Lactone	6.2	44.8 ± 1.6[*†]
Control	6.2	100

The values of cell viability are shown as the mean ± SD (%) relative to the respective control. [*]$p < 0.05$; Significantly difference from the respective control, [†]$p < 0.05$; Significantly difference from SN-38 Carbonate.
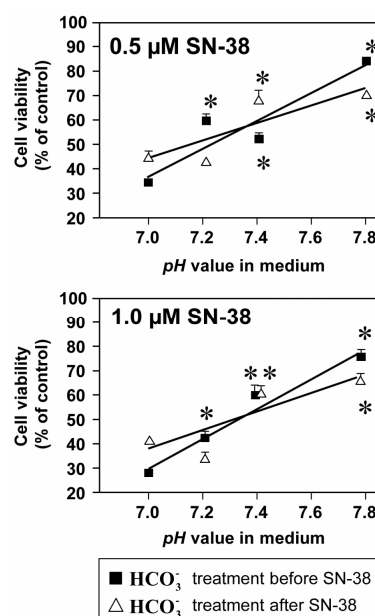
Figure 1. Effect of bicarbonate on SN-38-induced toxicity in HT29 cell. The cell was incubated with SN-38 (0.5 or 1.0 μM) for 2 h and increasing concentrations of bicarbonate (HCO_3^-; 5, 20, 44, and 100 mM) corresponding to *pH* 7.0, 7.2, 7.4, and 7.8, respectively, either for 2 h before (Closed squares) or after (Opened triangles) the SN-38 exposure. The cell in both conditions was further cultured in the medium with these increasing concentrations of HCO_3^- for additional 96 h. Cell viability was determined by the MTT assay. Data are shown as the mean ± SD of the percent of the respective control (0 μM SN-38 treatment). [*]Significantly different from the respective control determined in the presence of 5 mM bicarbonate, $p < 0.05$.

ured with increasing concentrations of HCO_3^- before to or after SN-38 exposures (0.5 or 1.0 μM) for 2 h. With HCO_3^- treatment before 0.5 μM SN-38 exposure, cell viability was significantly higher at *pH* 7.2, 7.4, and 7.8 than at *pH* 7.0, and at *pH* 7.8 was the highest. Similarly, with HCO_3^- treatment after 0.5 μM SN-38, cell viability at *pH* 7.4 and 7.8 was significantly higher compared to that at *pH* 7.0. Moreover, these characteristic patterns of viability seen in the 0.5 μM SN-38 were also observed in the exposure to 1.0 μM SN-38. There was no difference in SN-38-induced toxicity in the respective *pH* condition between HCO_3^- treatment before and after SN-38 exposures. The results indicate that HCO_3^- does not affect the conversion of SN-38 in the relatively short 2 h exposure period that is similar to small intestine transit time.

In addition, HT29 cell was incubated with the different HCO_3^- buffers for 4 h before and during 1.0 μM SN-38 lactone exposure. The cell was then washed and incubated in fresh DMEM containing 44 mM HCO_3^- (*pH*

7.4) for 96 h. Because the result showed that SN-38-induced toxicity was around 50% under all the different conditions (data not shown), it implied that HCO_3^- could not modify the influence of SN-38 on cell viability.
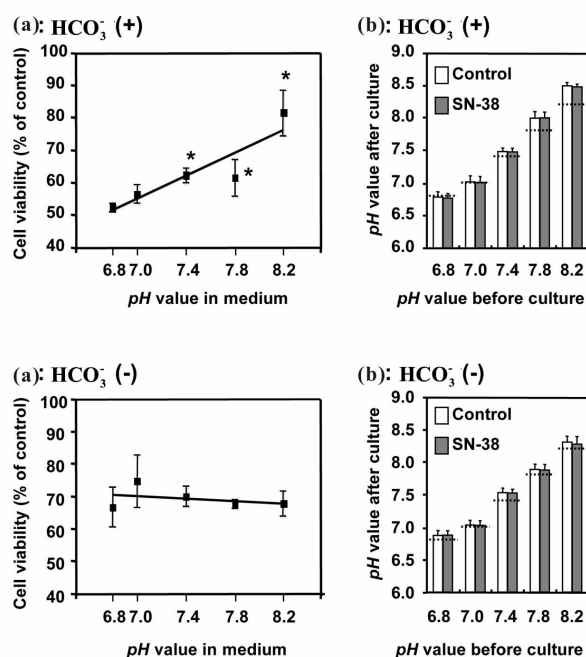
3.3. Effect of Bicarbonate-Adjusted *pH* on Cell Viability

Figure 2(a) shows the viability of HT29 cell treated with various *pH* conditions (6.8 - 8.2) adjusted by increasing concentrations of HCO_3^- or NaOH for 48 h following a 2 h SN-38 exposure. In the HCO_3^- treatment, the cell viability was significantly enhanced at *pH* 7.4, 7.8, and 8.2 compared to that at *pH* 6.8. On the other hand, there was no difference in the viability of HT29 cell treated without HCO_3^- among these *pH* conditions. In these experiments, the *pH* in the medium was almost perfectly maintained even after cell culture, and there was no difference in *pH* after culture between control and SN-38 exposure in both experiments (**Figure 2(b)**).

Figure 3 shows the flow cytometry image (**Figure 3(a)**) and the percentage of apoptotic cells analyzed by flow cytometry (**Figure 3(b)**) in the different *pH* conditions (48 h) with or without HCO_3^- following a 2 h exposure to 1 μM SN-38 lactone. In the control, the percentage of necrotic cells (double positive for annexin V-EGFP and PI) was higher at *pH* 6.8 with 5 mM HCO_3^- than at *pH* 7.8 with 44 mM HCO_3^- (**Figure 3(a)**). The necrosis at *pH* 6.8 was further aggravated by exposure to SN-38. Furthermore, the percentage of apoptotic cells (annexin V-EGFP positive) was increased. In the SN-38-exposed cells, the numbers of apoptotic and necrotic cells were lower at *pH* 7.8 than at *pH* 6.8. In the control cells, apoptotic cells were around 10% in all 3-set *pH* conditions despite of HCO_3^- treatment (**Figure 3(b)**). In case of SN-38-exposed cells, the apoptosis was enhanced by around 40% in all three *pH* conditions of HCO_3^- (−). On the other hand, the SN-38-induced apoptosis was significantly inhibited at *pH* 7.0 and 7.8 with increasing HCO_3^- concentrations compared to that at *pH* 6.8. These results show that higher *pH* conditions adjusted by HCO_3^- could inhibit the cellular toxicity induced by SN-38.

3.4. Effect of Bicarbonate on *pHi* Alternation Induced by SN-38

The level of *pHi* was determined in the presence of either 10 or 30 mM HCO_3^- following SN-38 (**Figure 4**). In the 10 mM HCO_3^-, *pHi* level in SN-38-exposed cells was significantly decreased compared to that in the control. On the other hand, there was no significant difference in the *pHi* between the control and SN-38-exposed cells in the presence of 30 mM HCO_3^-. Similar results
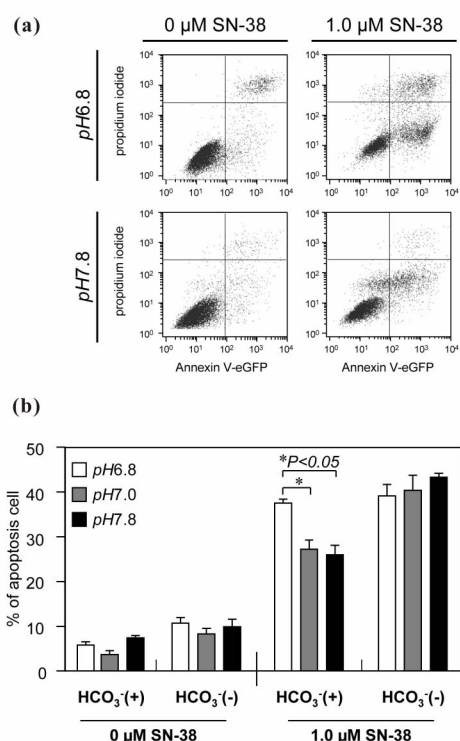

Figure 2. Effect of bicarbonate-adjusted *pH* on cell viability (a) and *pH* values in medium after cell culture (b). The cells incubated with/without 1.0 μM SN-38 for 2 h were cultured with *pH* set media at 5 points (6.8, 7.0, 7.4, 7.8, 8.2) adjusted by either different concentrations (5, 10, 20, 44, 100 mM, respectively) of bicarbonate [HCO_3^- (+)] or NaOH [HCO_3^- (-)]. (a) Cell viability evaluated by MTT assay was shown as the percent of the respective control (0 μM SN-38). *$p < 0.05$; Significantly different from *pH* 6.8; (b) The 5-set points of culture media adjusted by either increasing concentrations of bicarbonate [HCO_3^- (+)] or NaOH [HCO_3^- (−)] were measured after the cell culture. The dotted lines on each column show the set-*pH* values before the cell culture. The *pH* in each culture medium was measured using a standard *pH* meter in triplicate from three samples immediately after culture.

were obtained with a higher concentration of HCO_3^- (58 mM) as those obtained with 30 mM HCO_3^- (no data shown). The results indicate that higher concentrations of HCO_3^- could prevent intracellular acidification caused by SN-38.

4. Discussion

Diarrhea is one of the lethal major side effects in the cancer patients receiving CPT-11 [7,31]. We and others have previously demonstrated that intestinal alkalization by HCO_3^- could reduce CPT-11/SN-38-induced cell injuries and diarrhea in both *in vitro* and *in vivo* studies [12,13,15]. These studies were based on the role of HCO_3^- on intestinal *pH* regulation because of the *pH*-dependent cytotoxicity of CPT-11/SN-38 [1,9,10,12,14, 15,28,32,33]. In addition to the regulation of *pH* in the

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(a)

(b)

**Figure 3. Effect of bicarbonate-adjusted *pH* on apoptosis.
The cell following exposure to SN-38 was incubated for an
additional 48 h in 3-set *pH* conditions (6.8, 7.0, or 7.8) either
in HCO_3^- (+) or HCO_3^- (−) medium. See the legend of
Figure 2 for the bicarbonate concentrations that were used
to set the *pH* in the medium. Figure 3(a) shows the images
of flow cytometry in the cells exposed to either 0 or 1.0 μM
SN-38 lactone in HCO_3^- (+) conditions set at *pH* 6.8 or 7.8.
The apoptotic cells were expressed as the percentage of cells
stained with annexin V-EGFP positive and PI negative to
the total numbers of cells, and shown as the mean ± SD in
Figure 3(b).**

intestinal lumen, we suggested that HCO_3^- might play
other roles on cytoprotection against CPT-11/SN-38
through influence on *pHi* environment. Palissot *et al.*
have reported that the CPT-11/SN-38-induced apoptosis
was less in the multidrug-resistant HL60-Vinc leukemic
cells that could keep *pHi* higher, while apoptosis was
much in drug-sensitive HL60 cell which intercellular
acidification was induced following CPT-11/SN-38 treat-
ment [34]. Therefore, the regulation of *pHi* level would
be associated with the sensitive to CPT-11/SN-38 [18].

Accordingly, we designed a series of experiments in
the large intestine cell line to test the hypothesis that the
HCO_3^- might influence on not only extracellular *pH* but
also *pHi* environments, and consequently, disturb the
intracellular conversion of SN-38 from the less toxic
carboxylate form to the more toxic lactone form [9,35].
In the result, the cytotoxicity induced by SN-38 lactone
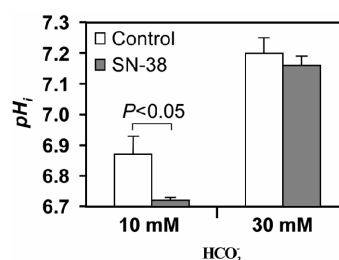
**Figure 4. Effect of bicarbonate on SN-38-induced change in
intracellular *pH*. Changes in *pHi* was assessed following
incubation of the HT29 cell in the presence and absence of
0.5 μM SN-38 for 2 h and in a culture medium containing
either 10 or 30 mM bicarbonate. The *pHi* was determined 4
h after removal of SN-38.**

form was attenuated in a dose-dependent matter of
HCO_3^-, but not by the respective pH condition merely
adjusted by NaOH/HCl with low concentration of
HCO_3^-. Although the HCO_3^- itself changes medium *pH*,
the result implies that the cell damages induced by SN-38
would depend on not only ambient *pH* environment be-
cause the exposed time to SN-38 was only 2 hours that is
too short to cause interconversion of SN-38 forms, and
therefore, HCO_3^- might play other roles on the protec-
tion against SN-38. One of the possibilities is that
HCO_3^- would be uptaked into cells, and then, would
affect the *pHi*. Indeed, the present study confirmed that a
higher concentration of HCO_3^- (30 mM) could inhibit
SN-38-induced intracellular acidification (**Figure 4**).

Furthermore, cellular acidification becomes a trigger
in the early phase of apoptosis because of activation of
endonucleases, and thus, regulation of *pHi* is critically
important for tumor development [21]. The present study
showed that the SN-38 itself has the action to cause in-
tercellular acidification in the intestinal cell. Besides the
inhibition of DNA topoisomerase I, it means that inter-
cellular SN-38 also behaves as a trigger of apoptosis.
Therefore, the regulation of *pHi* should be further im-
portant for attenuation of the side effects on CPT-11/
SN-38 therapy. In addition to the effect of HCO_3^- on
SN-38-induced intercellular acidification, Flow Cytome-
try assay showed that apoptosis following SN-38 expo-
sure was significantly decreased by increasing concentra-
tions of HCO_3^-, but not in the respective pH level ad-
justed by NaOH/HCl. These findings implied that the
regulation of *pHi* by HCO_3^- is effectiveness on the
prevention of SN-38 induced apoptosis in intestinal cells.

In addition to the interconversion of CPT-11/SN-38,
pH environment is also associated with other characteris-
tics of the agents. Gabr *et al.* have reported in L1210
leukemia cells that the uptake of radiolabeled CPT-11
was more rapidly at *pH* 6.2 than at physiological *pH* 7.4,
and the intercellular retention was predominant at *pH* 6.2

[36]. Similarly, we have previously reported that the up-take of SN-38 into HT29 cells was significantly increased at ambient lower *pH* [9]. In this point, HCO_3^- is indirectly contributed on the cellular uptake of SN-38 by regulation of ambient *pH* level.

In conclusion, the present study confirmed that HCO_3^- could keep alkalization/neutralization in not only extracellular but also intracellular *pH* environments, and consequently, cytotoxicity in large intestine cells would be attenuated through the reductions of interconversion to more toxic form and cellular uptake of SN-38. These results would certify the multiple roles of HCO_3^- on the attenuation of side effects; cell injuries and diarrhea, in CPT-11/SN-38 antitumor therapy.

5. Acknowledgements

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