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Influence of Non-Alcoholic Beverage on Gastrointestinal Epithelial Cells

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Original Research Article

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Abstract: When taking oral medications, it is recommended to take them with cold or warm water. However, if medication is taken regularly and the timing of ingestion is after meals, there are likely many cases in which the medicine was taken with fruit juice, tea, coffee, non-alcoholic drinks, and alcoholic drinks. The contents of non-alcoholic beverages are usually hidden and protected by law; therefore, unknown substances may impact the proliferation and function of gastrointestinal epithelial cells. The highest-selling non-alcoholic drink is Coca-Cola (2017), and its sales are third in Japan (2017). Therefore, drug intake by Coca-Cola is considered to be greater than that by other non-alcoholic drinks. In this study, to clarify the influence of Coca-Cola on gastrointestinal epithelial cells, we examined the influence of Coca-Cola on the proliferation and cellular uptake of HGC-27, IEC-6 and Caco-2, which are gastrointestinal epithelial cell lines. In the limited conditions (Concentration of Coca-Cola: 4%, Exposure time of Coca-Cola: 6 hr), significant changes in the proliferation of human gastrointestinal epithelial cells or uptake were not observed. Therefore, Coca-Cola was found to not significantly directly affect human gastrointestinal epithelial cells. However, although Coca-Cola does not influence human gastrointestinal epithelial cells, taking drugs with water is recommended.
Keywords: non-alcoholic beverage, cell proliferation, gastrointestinal epithelial cell, unstirred water layer

INTRODUCTION

When taking oral medications, cold or warm water is recommended. However, if medication is taken regularly and the timing of ingestion is after meals, there are likely many cases in which medicine was taken with fruit juice, tea, coffee, non-alcoholic drinks, and alcoholic drinks. Oral pharmaceutical products are developed assuming ingestion with water. Therefore, if drugs are taken with something other than water, interactions are possible, and many have been reported [1-3].

For example, when a medicine is ingested with an alcoholic beverage, the liver, which is the main metabolic organ, preferentially metabolizes the alcohol, delaying the metabolism of drugs, and the drugs thus remain in the body at a high concentration [4]. This is considered dangerous. Furthermore, as alcohol dehydrogenase (ALD) in the liver may change the components of drugs into harmful substances, there is a possibility of serious situations. Therefore, ingestion of drugs with alcoholic beverages, including beer, is considered to be extremely dangerous. Furthermore, when medications are taken with the combination of an antihypertensive agent and grapefruit juice [5, 6] or coffee [7], or antibiotics with milk [8, 9], there are risks of a difference in effects or side effects. In addition, caffeine in tea and coffee affects the action of medicines [10].

Soft drinks (non-alcoholic beverage) are a significant part of a normal diet. The ingredients of soft drinks are usually hidden and protected by law; therefore, unknown substances may impact the proliferation and function of gastrointestinal epithelial cells. The highest-selling non-alcoholic drink is Coca-Cola (2017)[11], and its sales are the third in Japan(2017) [12]. Therefore, drug intake by Coca-Cola is considered to be greater than that by other non-alcoholic drinks.

Nowacki *et al.* reported the proliferative effects of Coca-Cola on NIH/3T3 fibroblasts [13]. Of note, differences in effects depended on the country in that manuscript. As some information for soft drinks is protected by companies (patents), the product details are not disclosed. Although the mechanism of the proliferation-increasing effects by Coca-Cola is unknown, Coca-Cola does indeed affect cells. Moreover, that report did not consider the impact of

drinking Coca-Cola, and even though beverages are first exposed to gastrointestinal epithelial cells after ingestion, such cells were not used. Therefore, in this study, we aimed to clarify the influence of Coca-Cola on gastrointestinal epithelial cells.

MATERIALS AND METHODS

Materials

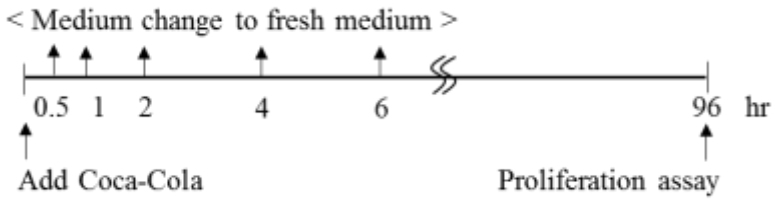
Crystal Violet and Rose Bengal were purchased from Wako Pure Chemical Industries. Ltd. (Osaka, Japan). All other reagents were of analytical grade or higher.

Cell culture

HGC-27, IEC-6, and Caco-2 cells were obtained from Riken Cell Bank (Ibaraki, Japan) and kept in a humidified incubator at 37°C with 5% CO_2. HGC-27 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM)-High glucose (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, 100 mg/ml of streptomycin and 250 ng/ml of amphotericin B, and were used in passages 9 - 11. Caco-2 cells were maintained in DMEM-High glucose (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 100 U/ml of penicillin, and 100 mg/ml of streptomycin, and were used between passages 9 - 10. IEC-6 cells were maintained in DMEM-High glucose (Wako, Osaka, Japan) supplemented with 5% fetal bovine serum, 4 μg/ml of insulin, 100 U/ml of penicillin, 100 mg/ml of streptomycin and 250 ng/ml of amphotericin B, and were used between passages 4 - 5.

Proliferation assay

For the growth assay, each cell line was seeded onto 96 well culture plates (HGC-27 and IEC-6: 2.0×10^3 cells/well, Caco-2: 1.0×10^4 cells/well), and cultured for 24 hr to adhere to the culture plate. Thereafter, the medium was replaced with fresh medium with/without each concentration of Coca-Cola and incubated for 0.5 to 6 hr. After each required time had elapsed, medium was exchanged, and cells were cultured for 96 hr (Scheme-1).



Scheme-1: Time course of Coca-Cola administration for the proliferation assay

To measure cell proliferation, relative cell numbers were measured using Crystal Violet staining for adherent cells. Cells were fixed with 4% paraformaldehyde in PBS for 10 min, and stained with 0.04% Crystal Violet aqueous solution for 15 min and dissolved in 1% SDS. In the end, the cell viability was estimated by measuring the absorbance on a microplate reader (ARVO MX 1420 MULTILABEL COUNTER, PerkinElmer Inc., MA, USA) at 560 nm.

Uptake experiment

For the uptake experiment, each cell lime was seeded onto 24 well culture plates (1.0×10^5 cells/well), and cultured for 24 hr to adhere to the culture plate. Thereafter, the medium was replaced with fresh medium with/without each concentration of Coca-Cola and incubated for 2 hr. Before starting the uptake experiment, cells were rinsed with Hank's Balanced Salt Solution + 10 mM HEPES buffer (0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 6 mM glucose, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$ and 1.0 mM $MgSO_4$) 2 times and pre-incubated with it for 15 min at 37°C. Thereafter, the pre-incubation buffer were removed and Rose Bengal including buffer was added. After the uptake experiment, cells were rinsed with ice-cold PBS 3 times and dissolved in 0.1% triton-x. Then, the uptake of Rose Bengal was estimated by measuring the absorbance on a microplate reader (ARVO MX 1420 MULTILABEL COUNTER, PerkinElmer Inc., MA, USA) at 530 nm.

Statistical analysis

All results are expressed as the mean ± standard error (S.D.). The significance between groups was analyzed using the Student's t-test; $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Influence of Japanese Coca-Cola on Gastric Epithelial Cells (HGC-27)

Alcohol is known to cause damage to the gastric epithelium independently of gastric acid secretion. However, that mechanism has not been systematically clarified. Moreover, it is not known whether the non-alcoholic components of alcoholic beverages play a role in the pathogenesis of gastric epithelial cell damage. Therefore, non-alcoholic beverages may influence gastric epithelial cells. Thus, in this study, we examined the influence of Coca-Cola on HGC-27, a human gastric cancer cell line.

As the pH of the stomach is around 2, we examined the effects of Coca-Cola on the proliferation of HGC-27 at pH 2. The concentration of Coca-Cola

was 2% or 4%. In addition, the exposure time to 4% Coca-Cola by HGC-27 was 0.5, 1, 2, 4 or 6 hr, and the growth assay was performed 96 hr after the administration of Coca-Cola (Scheme-1). In Fig-1, the proliferation rate of HGC-27 slightly changed (Fig-1a), but there was no significant difference between the presence and absence of Coca-Cola for all exposure times (Fig-1b). Thus, the proliferation ratio of HGC-27 did not significantly change with 4% Coca-Cola (Fig-1). Moreover, no significant change was observed in proliferation rates of HGC-27 even when higher concentrations (up to 20%) were applied (data not shown).

In general, the cellular uptake of some compounds occurs via transcellular permeation. The permeability via the transcellular route is limited by the unstirred water layer (UWL), which consists of mucin. To clarify the influence of Coca-Cola on the gastric epithelial membrane surface, the uptake of Rose Bengal, which is affected by UWL, was examined. The conditions of uptake experiment were set at pH 2 or pH 7.4. In both conditions, the uptake rates and amounts of Rose Bengal were not changed by exposure to Coca-Cola (Fig-1c, 1d), demonstrating that Coca-Cola has no effects on the mucin layer (UWL) of gastric epithelial cells. From these results, Coca-Cola has no significant influence on HGC-27 cells.

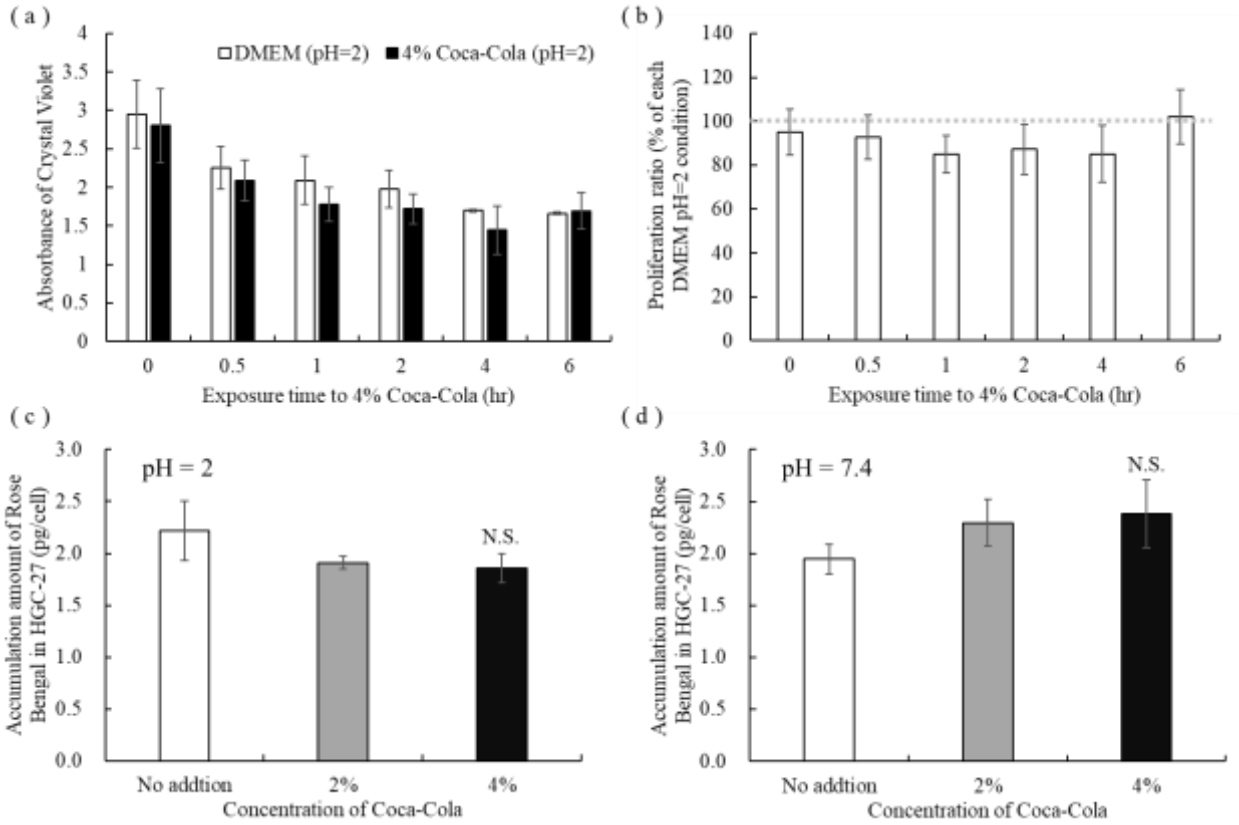


Fig-1: Effects of Coca-Cola on cell proliferation (a and b) and uptake of Rose Bengal (c and d) of HGC-27.
Data represent means and S.D. (n = 3 - 9 for each condition)

Influence of Japanese Coca-Cola on Small Intestinal Epithelial Cells (IEC-6)

Next, we examined the influence of Coca-Cola on small intestinal epithelial cells using IEC-6, which is a mouse small intestinal epithelial cell line. Coca-Cola is highly acidic (pH range 2 - 3) due to carbonation, citric acid and phosphoric acid. Although Coca-Cola has a relatively high buffering capacity, it is expected to be neutralized by saliva and the components in the digestive tract. Therefore, the media containing Coca-Cola was neutralized to pH 7.4 for the proliferation assay of IEC-6.

The proliferation rate of IEC-6 was significantly inhibited in dose- and exposure time-dependent manners (Fig-2a). Although the proliferation of IEC-6 was significantly inhibited by exposure to Coca-Cola, the uptake of Rose Bengal was not changed by exposure to 2% or 4% Coca-Cola (Fig-2b). As such, Coca-Cola did not affect the drug uptake in these experimental conditions, but it inhibited the proliferation of IEC-6.

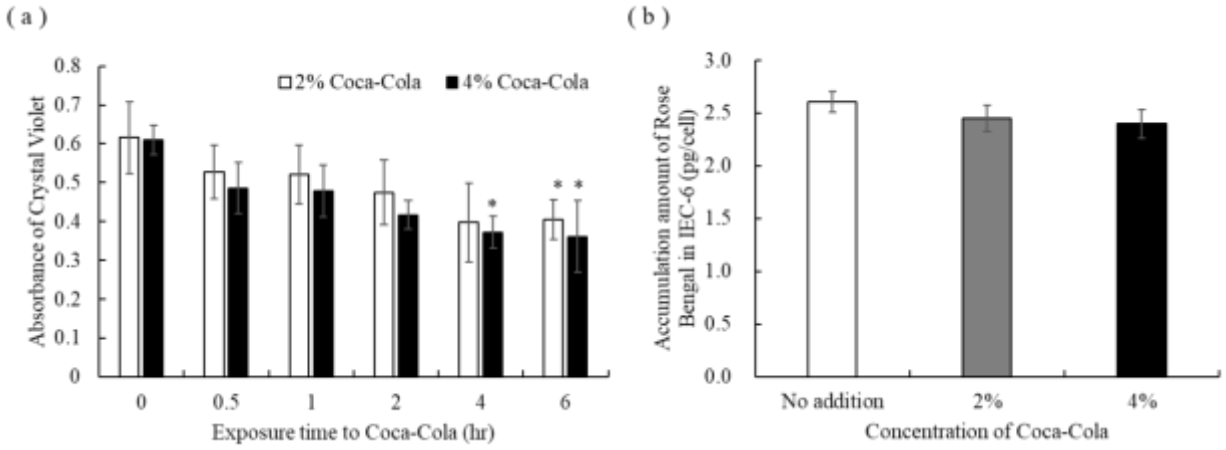


Fig-2: Effects of Coca-Cola on cell proliferation (a) and uptake of Rose Bengal (b) by IEC-6.
Data represent means and S.D. (n = 3 - 9 for each condition) *P < 0.05 vs. 0 hr condition.

Influence of Japanese Coca-Cola on Colorectal Epithelial Cells (Caco-2)

Lastly, we examined the influence of Coca-Cola on colorectal epithelial cells using Caco-2, a human colorectal cancer cell line. As for IEC-6, the media containing Coca-Cola was neutralized to pH 7.4 for the Caco-2 experiments.

With an exposure time of up to 6 hr, the proliferation rates of Caco-2 were not significant changed by exposure to 2% or 4% Coca-Cola (Fig-3a). Furthermore, the uptake of Rose Bengal was not changed by exposure to 2% or 4% Coca-Cola (Fig-3b).

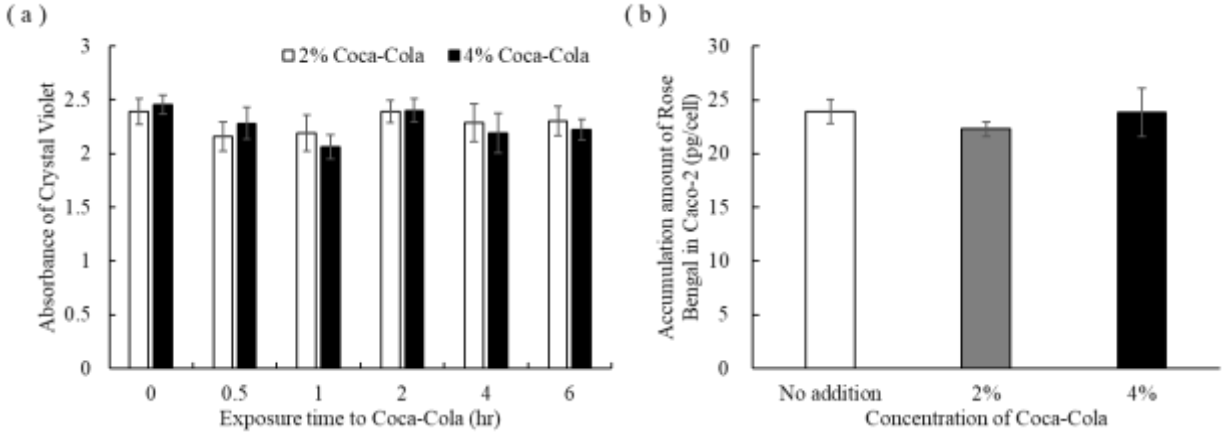


Fig-3: Effects of Coca-Cola on cell proliferation (a) and uptake of Rose Bengal (b) by Caco-2.
Data represent means and S.D. (n = 3 - 9 for each condition)

Although Japanese Coca-Cola had almost no effect on human gastrointestinal epithelial cells, these data were under limited conditions (Concentration: 2 or 4%, Exposure time: 0.5 – 6 hr). Thus, it cannot be concluded that Japanese Coca-Cola does not affect human gastrointestinal epithelial cells, and further examination under more conditions is necessary to clarify the influence of Japanese Coca-Cola on gastrointestinal epithelial cells. Moreover, this study used only Classic Coca-Cola, and similar products such as Coca-Cola Zero and Diet Coca-Cola, which have high sales like Classic Coca-Cola, should be studied.

On the other hand, the proliferation of IEC-6 was significantly inhibited by exposure to Coca-Cola (Fig-2a), but HGC-27 and Caco-2 were not affected (Fig-1a, 3a). It is unknown why only the proliferation of IEC-6 was inhibited. One possibility is that IEC is of mouse origin comprising a small intestine cell line and normal cell line. In another report, the proliferation of rat embryonic fibroblasts (REF) was significantly inhibited by exposure to energy drinks [14]. Thus, non-alcoholic beverages may have rodent-specific activity. Moreover, our results were not consistent with those of Nowacki *et al.*, Regarding this inconsistency, differences in cell types and producing countries of Coca-Cola are possible explanations. Further studies are necessary to clarify the mechanism of this phenomenon caused by Coca-Cola.

This study examined the interaction between Coca-Cola and gastrointestinal epithelial cells. In the

future, a study on the interaction between Coca-Cola and pharmaceutical drugs is needed. The interaction among Coca-Cola, gastrointestinal epithelial cells and pharmaceutical drugs is important.

CONCLUSION

Under limited conditions (concentration of Coca-Cola: 4%, Exposure time: until 6 hr), no significant changes in the proliferation of human gastrointestinal epithelial cells or uptake of a compound were observed. Therefore, Coca-Cola has no significant direct effects on human gastrointestinal epithelial cells. However, as this study was performed under limited conditions, a more detailed examination is necessary to clarify the association between Coca-Cola and the solubility of pharmaceutical products. Although Coca-Cola does not influence human gastrointestinal epithelial cells, taking drugs with water is recommended.

CONFLICT OF INTEREST (COI)

The authors declare no conflict of interest.

REFERENCES

1. Bailey DG. Fruit juice inhibition of uptake transport: a new type of food–drug interaction. British journal of clinical pharmacology. 2010 Nov 1;70(5):645-55.
2. Oda K, Murakami T. Pharmacokinetic interaction of green tea beverage containing cyclodextrins and high concentration catechins with P-glycoprotein substrates in LLC-GA5-COL150 cells in vitro and in the small intestine of rats in vivo. Journal of Pharmacy and Pharmacology. 2017 Dec 1;69(12):1736-44.
3. Dresser GK, Urquhart BL, Proniuk J, Tieu A, Freeman DJ, Arnold JM, Bailey DG. Coffee inhibition of CYP3A4 in vitro was not translated to a grapefruit-like pharmacokinetic interaction clinically. Pharmacology research & perspectives. 2017 Oct 1;5(5).
4. Tanaka E, Misawa S. Pharmacokinetic interactions between acute alcohol ingestion and single doses of benzodiazepines, and tricyclic and tetracyclic antidepressants–an update. Journal of clinical pharmacy and therapeutics. 1998 Oct 1;23(5):331-6.
5. Jáuregui-Garrido B, Jáuregui-Lobera I. Interactions between antihypertensive drugs and food. Nutricion hospitalaria. 2012;27(6).
6. Uesawa Y, Takeuchi T, Mohri K. Publication bias on clinical studies of pharmacokinetic interactions between felodipine and grapefruit juice. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2010 May 1;65(5):375-8.
7. Bailey DG, Dresser GK, Urquhart BL, Freeman DJ, Arnold JM. Coffee—Antihypertensive Drug Interaction: A Hemodynamic and Pharmacokinetic Study With Felodipine. American journal of hypertension. 2016 Dec 1;29(12):1386-93.
8. Lehto P, Kivistö KT. Different effects of products containing metal ions on the absorption of lomefloxacin. Clinical Pharmacology & Therapeutics. 1994 Nov 1;56(5):477-82.
9. El-Sooud KA. Influence of albendazole on the disposition kinetics and milk antimicrobial equivalent activity of enrofloxacin in lactating goats. Pharmacological research. 2003 Oct 1;48(4):389-95.
10. Bjørkedal E, Flaten MA. Interaction between expectancies and drug effects: an experimental investigation of placebo analgesia with caffeine as an active placebo. Psychopharmacology. 2011 Jun 1;215(3):537-48.
11. Food Inc. on NBC NEWS.com, Available from http://www.nbcnews.com/id/42255151/ns/business-us_business/t/sweet-americas-top-brands-soda/#.WsxS1S7FLIU
12. TOYO KEIZAI ONLINE, Available from https://toyokeizai.net/articles/-/186673?page=2
13. Nowacki M, Adamowicz J, Olkowska J, Pietkun K, Kloskowski T, Bajek A, Drewa T. Non-alcoholic beverages, unknown influence on cell proliferation-an in vitro study. Annals of Agricultural and Environmental Medicine. 2014;21(1).
14. Doyle W, Shide E, Thapa S, Chandrasekaran V. The effects of energy beverages on cultured cells. Food and chemical toxicology. 2012 Oct 1;50(10):3759-68.