Title	Diet supplementation with cholic acid promotes intestinal epithelial proliferation in rats exposed to γ -radiation
Author(s)	Hagio, Masahito; Shimizu, Hidehisa; Joe, Ga-Hyun; Takatsuki, Manami; Shiwaku, Maiko; Xu, Hong; Lee, Ja-Young; Fujii, Nobuyuki; Fukiya, Satoru; Hara, Hiroshi; Yokota, Atsushi; Ishizuka, Satoshi
Citation	Toxicology Letters, 232(1), 246-252 https://doi.org/10.1016/j.toxlet.2014.10.011
Issue Date	2015-01
Doc URL	http://hdl.handle.net/2115/81755
Rights	© 2014. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	http://creativecommons.org/licenses/by-nc-nd/4.0/
Type	article (author version)
File Information	Toxicology letters_232_246.pdf ()

[Instructions for use](#)

Diet supplementation with cholic acid promotes intestinal epithelial proliferation in rats exposed to γ -radiation

Masahito Hagio ^{a,b,1}, Hidehisa Shimizu ^{a,1}, Ga-Hyun Joe ^a, Manami Takatsuki ^a, Maiko Shiwaku ^a, Hong Xu ^{a,c}, Ja-Young Lee ^a, Nobuyuki Fujii ^a, Satoru Fukiya ^a, Hiroshi Hara ^a, Atsushi Yokota ^a, and Satoshi Ishizuka ^{a,*}

^a Division of Applied Bioscience, Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

^b Faculty of Life Sciences, Toyo University, Ora 374-0193, Gunma, Japan.

^c School of Food Science and Chemical Engineering, Beijing Technology and Business University, Beijing 100048, China

* Corresponding author. Address: Division of Applied Bioscience, Research Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589, Japan

E-mail address: zuka@chem.agr.hokudai.ac.jp (S. Ishizuka).

¹ These authors contributed equally to this paper.

Abstract

Consumption of a high-fat diet increases some secondary bile acids (BAs) such as deoxycholic acid (DCA) in feces. DCA is derived from cholic acid (CA), a primary BA. We evaluated intestinal epithelial proliferation and BA metabolism in response to oral administration of cholic acid (CA) in rats to determine the influence of a CA diet on the responses of gut epithelia to γ -rays. WKAH/HkmSlc rats were divided into two dietary groups: control diet or CA-supplemented (2 g/kg diet) diet. Some of the rats from each group were irradiated with γ -rays, and epithelial cell proliferation in the colon was analyzed histochemically. Unirradiated CA-fed rats had high levels of DCA and CA in the sera, as well as the presence of taurocholic acid in their feces. Significant increases were observed in both epithelial proliferation and the number of epithelial cells in the colon of the CA-fed rats, and this effect was observed at 8 weeks after γ -ray exposure. Furthermore, extracts from both cecal contents and sera of the unirradiated CA-fed rats promoted proliferation of IEC-6 cells. These results indicate that BAs in enterohepatic circulation promote proliferation and survival of the intestinal epithelium after receiving DNA damage.

Keywords:

Bile acid

Intestinal epithelial kinetics

γ -rays

Ultra performance liquid chromatography-electrospray ionization mass spectrometry

Cecal contents

IEC-6 cells

1. Introduction

The morbidity of colorectal cancer (CRC) is increasing, and CRC mortality is approximately 33% in the developed world (Center et al., 2009). The pathogenesis of CRC is correlated with obesity (Walther et al., 2009). Obese patients often have enhanced bile secretion (Reddy, 1981), and bile acid (BA) has been identified as a risk factor for the pathogenesis of CRC (Degirolamo et al., 2011). BAs contribute to digestion and absorption of dietary lipids, including lipophilic vitamins, in the gut. Cholic acid (CA), a main component of primary BA (PBA), is hepatically synthesized from cholesterol in humans and rodents. Certain secreted BAs are reabsorbed from the distal ileum and reused, the non-recycled PBAs are converted into secondary BA (SBA) by the intestinal bacteria in the large intestine. In humans and rodents, deoxycholic acid (DCA), one of the SBAs, is produced from CA. DCA is a carcinogenic agent and has been implicated as a promoter of CRC (Bernstein et al., 2011).

To study the relationship between BAs and CRC, the occurrence of aberrant crypt foci (ACF) is widely used as a biomarker of CRC. This biomarker has been used in multiple studies because ACF resemble adenomas on the mucosal surface and are associated with abnormal epithelial proliferation (Raju, 2008; Magnuson et al., 1993; Lin et al., 2001). However, experiments to evaluate ACF were not successful in clarifying the role of BAs in CRC development because the number of ACF does not necessarily reflect subsequent CRC development. Mucin-depleted foci (MDF) are another biomarker and considered as more dysplastic lesions than ACF due to integrity of the nuclear outline and mucin depletion (Caderni et al., 2003, Ochiai et al., 2014). Rats fed a CA-supplemented diet and treated with the colon-carcinogen azoxymethane exhibit increased frequency and multiplicity of MDF (Femia et al., 2004). Then, we focused on DNA damage because accumulation of multiple DNA mutations in the normal colonic epithelium leads to carcinoma formation (Takayama et al., 2006). In addition, γ -irradiation (4 Gy) induces responses similar to those induced by chemical carcinogens in the epithelial cells (Ishizuka et al., 2003). We used epithelial kinetics as a biomarker to examine the epithelial response to BA, during the initiation stage of CRC.

Because CA secretion is increased in high-fat diet-induced obesity and aging, we evaluated the influence of a CA-supplemented diet on BA metabolism in addition to examining colonic epithelial kinetics and crypt survival in rats exposed to γ -rays. Furthermore, we determined the effects of intestinal extracts and sera on IEC-6 cell

proliferation from rats fed either a CA-supplemented or control diet.

2. Materials and Methods

2.1. Animals

The study was approved by the Hokkaido University Animal Committee. All animals were maintained in accordance with Hokkaido University guidelines for the care and use of laboratory animals. Male 3-week-old WKAH/Hkm Slc rats (Japan SLC, Shizuoka, Japan) were used throughout this study. The rats were housed in an air-conditioned room at $22 \pm 2^{\circ}\text{C}$ with $55 \pm 5\%$ humidity, and the light period was from 8:00 to 20:00. The rats were housed individually in standard wire-bottomed cages and allowed free access to food and water. During the 3-day acclimation period, the rats were fed a control diet based on the AIN-93G formulation (Reeves et al., 1993) shown in Table 1. For the CA-supplemented diet, 2 g of CA /kg was added to the control diet at the expense of sucrose.

2.2. Influence of the CA diet on rat growth parameters and BA metabolism

After acclimation, the rats (n=12) were divided into two groups and were fed either the control (n=6) or CA diet (n=6) for 10 days. At the end of the test period, the rats were deprived food for 3 h to avoid the influence of feeding on BA concentration and composition (Schlam et al., 1978) and sacrificed. The rats were anesthetized using a ketamine and xylazine mixture containing 50 mg/ml ketamine and 8.64 mg/ml xylazine (60 μl /100 g body weight), and the abdominal cavity was opened. Arterial blood was collected from the aorta abdominalis. The sera were obtained immediately after collection by centrifugation at $1,000 \times g$ for 10 min at 4°C . Liver, kidney, small intestine, cecum, colorectum, mesenteric adipose tissue, perirenal with dorsal adipose tissue, and epididymal adipose tissue were collected, and their weights were measured. Feces were collected at the end of the test period and stored at -80°C until analysis.

2.3. Crypt survival and epithelial mitosis in the rat colon at 4 days after γ -ray exposures

After the acclimation period, forty rats were fed either the control or the CA diet (n=20/diet) for 10 days. Each group of rats was further separated into 5 groups and exposed to γ -rays at 0, 1, 4, 8, or 10 Gy. The exposure was performed between 10:00 and 12:00. The γ -ray source was a ^{60}Co γ -irradiator (cobalt-60 teletherapy apparatus

RCR-12-C3; Toshiba, Kanagawa, Japan). The dose rate of the whole body exposure to γ -rays was 0.65 Gy/min. Four days after γ -ray exposure, the distal colon was collected under sodium pentobarbital anesthesia (Nembutal, 35 mg/kg body weight; Abbott Laboratories, Abbott Park, IL, USA).

2.4. Colonic epithelial kinetics in response to a sublethal dose of γ -rays

After the acclimation period, rats were fed either the control diet or the CA diet for 10 days and subsequently exposed to γ -rays (4 Gy) as described above. One hour before euthanasia at 0, 1, 3, 6, 12, and 24 h after irradiation (n=6 each), the rats were subcutaneously injected with bromodeoxyuridine (BrdU, 15 mg/kg body weight) as described previously (Ishizuka et al., 2003). All rats were sacrificed under sodium pentobarbital anesthesia, and the distal colon was collected.

2.5. Epithelial proliferation in the rat colon at 8 weeks after exposure to a sublethal dose of γ -rays

Groups of 5 rats were fed either the control or the CA diet for 10 days before abdominal exposure to γ -radiation (4 Gy) as described above. The animals were fed the same diet for another 8 weeks, after which all rats were sacrificed under sodium pentobarbital anesthesia, and the distal colon was collected.

2.6. BA analysis

The amount of each BA in the feces and sera were extracted and measured as previously reported (Hagio et al., 2009). The individual BA concentrations were measured with nordeoxycholic acid (23-nor-5 β -cholanic acid-3 α ,12 α -diol) (Steraloids, Inc., Newport, RI, USA) as the internal standard.

2.7. Histochemical analysis

After removing the colon, the intestinal contents were washed out with cold saline, and the colon tissues were fixed with 10% formalin in PBS. The preparation of the tissue sections and the tissue assessment was performed as described in our previous paper (Ishizuka et al., 2003). BrdU-incorporating cells, apoptotic cells, and mitotic cells were scored on a cell-positional basis within the crypts according to the method of Ijiri and Potten (1983). In brief, fifty-half crypts were counted from each individual rat.

Apoptosis assessment relied on morphological characteristics, such as cell shrinkage, chromatin condensation, and cellular fragmentation (Kerr et al. 1972).

2.8. Cell culture and proliferation assay

IEC-6 cells, a normal rat intestinal epithelial cell line, were obtained from the American Type Culture Collection (Manassas, VA. USA). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM, 12100-046; Gibco, Invitrogen Co., Carlsbad, CA. USA) supplemented with 5% heat-inactivated fetal bovine serum, 0.1 U/mL insulin, 35 µg/mL penicillin G potassium (Wako Pure Chemical Industries, Ltd. Osaka, Japan), and 100 µg/mL streptomycin sulfate (Wako Pure Chemical Industries, Ltd. Osaka, Japan) and were cultured at 37°C in a humidified atmosphere with 95% air and 5% CO₂. IEC-6 cells were seeded at 1.5×10^3 cells/well in 96-well plates and incubated in DMEM for 24 h. Then, the cells were exposed to DMEM containing the heat-inactivated sera (2%) or the cecal extracts from the unirradiated rats fed the control or CA diet. The cecal contents were diluted with 4 times their volume of deionized water, and the homogenates were further diluted with DMEM (x 5,000) before use. Cell counts were measured using a cell counting kit -8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's guidelines. The optical density of the culture supernatant was determined at 450 nm for measurement and 655 nm for reference.

2.9. Statistics

Statistical significance for comparison of control and CA-supplemented diet-fed groups was determined using Student's *t*-test. Two-way ANOVA was performed to analyze epithelial status, epithelial kinetics, and IEC-6 proliferation. For histological scoring at each cell position between BrdU incorporating cells in the unirradiated rats and apoptotic index in the irradiated rats, the data were analyzed by modified median test according to Potten (1990). A probability of less than 0.05 was considered statistically significant. JMP version 11.0 (SAS Institute Inc., Cary, NC) was used for the statistical analyses.

3. Results

3.1. Growth parameters and bile acid profiles

The various parameters and tissue weights are shown in Table 2. At day 10,

multiple growth parameters (body weight, total food intake, and the weights of each type of adipose tissue) were decreased significantly in the rats fed the CA diet compared with those in the control rats. In contrast, the amount of bile flow was significantly increased in the rats fed the CA diet. Composition and distribution analysis of BAs is utilized to understand precise BA metabolism because CA can be easily metabolized and converted to other types of BAs. In the CA-fed rats, both DCA and CA increased prominently. Taurine-conjugated CA (TCA), α -muricholic acid (T α MCA), DCA (TDCA), glycine-conjugated CA (GCA) and DCA (GDCA) were also observed in the feces of the CA-fed rats (Table 3). The CA-fed rats had levels of total BA (TBA) and the BA excretion per day that were approximately 13-fold higher and 12-fold higher, respectively, than the control rats. A 11-fold increase in serum TBA was observed in the CA-fed rats (Table 4). The CA-fed rats had concentrations of CA and TCA that were 24- and 7.8-fold higher, respectively, than those in the control rats.

3.2. Crypt survival and mitosis in the colon after exposure to γ -rays

Fig. 1 shows that the ingestion of CA influenced crypt survival and epithelial mitosis in the distal colon. The number of colon crypts remaining after irradiation was higher in the CA-fed rats than that in the irradiated controls (Fig. 1A). This trend was confirmed by quantitation of epithelial mitosis (Fig. 1B).

3.3. Epithelial cell kinetics after γ -irradiation

Fig. 2 shows the acute kinetics of colonic epithelia in the rats exposed to γ -rays. As expected, the CA diet increased proliferation-related parameters such as BrdU incorporation (Fig. 2A) and mitosis (Fig. 2B) in the unirradiated rats. The γ -irradiation modulated these epithelial proliferation parameters after irradiation and the patterns were very similar in both dietary groups. There was a reduction in BrdU-incorporation and mitosis during the experimental period. In contrast, the apoptotic index increased transiently at 3 or 6 h after the exposure (Fig. 2C). To evaluate the balance between proliferation and apoptosis at each cell position in the colonic crypts, the rates of BrdU incorporation in the unirradiated rats were compared with the apoptotic index at 3 hours after γ -ray irradiation (Fig. 2D). In the colon of control rats, apoptosis rates appeared to be greater than the proliferation rates. In contrast, the level of BrdU incorporation appeared to be higher than the apoptotic index in the CA-fed rats, especially in the

middle region of the crypts. These results indicate that a CA diet promotes the survival of proliferating epithelial cells exposed to γ -rays.

3.4. IEC-6 proliferation in response to cecal extracts and sera from the CA-fed rats

We questioned whether some component of the colonic environment in the CA-fed rats influences epithelial proliferation. To evaluate this possibility, IEC-6 cells were incubated with a medium containing extracts of either the cecal contents or sera from the CA-fed rats (Fig. 3). Exposure to the cecal content extracts and sera from rats fed the CA-diet results in an increase in the number of IEC-6 cells compared to cecal content extracts and sera from the control rats. Thus, BA or other metabolites in the colonic environment are involved in the promotion of epithelial cell proliferation in CA-fed rats.

3.5. Epithelial proliferation in the rat colon 8 weeks after exposure to a sublethal dose of γ -rays

Colonic epithelial proliferation was examined in the rats fed the CA diet at 8 week-post-exposure to γ -ray (Fig. 4). Increased BrdU incorporation in CA-fed rats was still observed 8-week after exposure.

4. Discussion

Several novel findings are detailed in the present study: 1) BA metabolism in the CA-fed rats was quite abnormal; 2) colonic epithelial proliferation was increased in rats fed the CA diet; 3) the increase in epithelial proliferation was maintained even after exposure to a sublethal dose of γ -rays; and 4) the cecal extracts and sera of the CA-fed rats promoted IEC-6 cell proliferation. Taken together, the results suggest that abnormal BA metabolism is involved in the promotion of colonic epithelial proliferation regardless of γ -ray exposure.

Cell kinetic analysis shows the distribution of the cells in proliferation, mitosis, and apoptosis at each cell position. In Fig. 2D, apoptosis rates in rats exposed to γ -rays almost equaled the rates of proliferations measured by BrdU-incorporating cells, suggesting that almost all the epithelial cells that received γ -radiation underwent apoptosis because intestinal epithelial cells in the proliferating region are very sensitive against radiation (Ijiri and Potten, 1983). In contrast, in the rats fed CA diet, the

distribution of BrdU-incorporating cells far exceeded apoptosis rates. This result indicates that CA-fed rats had increased proliferation, even after received γ -radiation. In the colon, stem cells are located at the bottom of the crypts. Fig. 2D also shows that the colonic epithelial stem cells in CA-fed rats were selectively eliminated in response to γ -rays although other proliferating cells were still alive. Presumably, such differences might be in relation to the propagation of dysplastic epithelial cells such as MDF in CA-fed rats in the later time points (Femia et al., 2004).

In the literature, BAs are suggested to increase energy consumption and decrease lipid synthesis, thereby inducing loss of body weight and suppressing fat accumulation via activation of TGR5 and FXR (Houten et al., 2006). In addition, TGR5 activation by BAs and rectal administration of TCA induce secretion of glucagon-like peptide (GLP-1) (Katsuma et al., 2005; Wu et al., 2013). It is possible that CA ingestion might suppress the appetite and food intake of rats through GLP-1 secretion in the present study. However, such a reduction in food intake by BAs appears to be artificial based on the BA distribution pattern and the fact that a reduced level of CA supplementation in the diet prevents the effect (Islam et al., 2011).

BA deconjugation by intestinal bacteria is almost complete in the cecum and colorectum under normal physiological conditions (Hagio et al., 2009). In the present experiment, the CA deconjugation was incomplete in the CA-fed rats as shown in supplemental Table 3. CA supplementation might also inhibit utilization of ω -MCA, a 6 β -epimerized metabolite synthesized from β -MCA (Sacquet et al., 1979; Eyssen et al., 1983), by the intestinal bacteria. Dietary supplementation of CA beyond 0.2% seems to be enough to alter bacterial BA metabolism in the gastrointestinal tract. The massive increase in CA in the gastrointestinal tract may select for BA-resistant bacteria, whereas some other bacteria may be eliminated by the antibacterial effect of CA and CA-related BAs such as DCA (Islam et al., 2011).

TGR5 is highly expressed in the mouse ileum and colon and is strongly activated by DCA (Maruyama et al., 2006; Sato et al., 2008). It is possible that over-production of DCA following CA ingestion in both the gastrointestinal tract and serum induces colonic epithelial proliferation. However, studies have also shown that DCA-induced activation of TGR5 leads to apoptosis in hepatocytes (Yang et al. 2007) and DCA elicits apoptosis in IEC-6 cells (Ishizuka et al., 2012). Based on these results, activation of TGR5 and over-production of DCA might not affect epithelial proliferation of colon

cells in CA-fed rats. Recently, TCA, TDCA, TUDCA, GCA, and GDCA were shown to activate sphingosine-1-phosphate receptor 2 (S1PR2), but not sphingosine-1-phosphate receptors 1 and 3, in cholangiocarcinoma cells and primary rodent hepatocytes (Studer et al., 2012; Liu et al., 2014). We showed previously that IEC-6 cell proliferation is promoted by the addition of some conjugated BAs (Ishizuka et al., 2012), but not by DCA. In the present study, high concentrations of TCA and TDCA were observed in both the feces and sera of the CA-fed rats. Thus, TCA- and TDCA-activated S1PR2-related signaling pathways may be involved in cell proliferation in the rat colon, and both cecal content extracts and sera induced IEC-6 cell proliferation.

In conclusion, the CA-supplemented diet causes dysregulation of BA metabolism and stimulates colonic epithelial proliferation even after DNA damage by γ -irradiation. Controlling BA metabolism via dietary changes may contribute to the prevention of the initiation and progression of CRC.

Acknowledgments

This study was supported by the Regional Innovation Strategy Support Program of the MEXT (Ministry of Education, Culture, Sports, Science and Technology) from the Japanese government, and Yakult Bio-Science Foundation.

References

- Bernstein, C., Holubec, H., Bhattacharyya, A.K., Nguyen, H., Payne, C.M., Zaitlin, B., Bernstein, H., 2011 Carcinogenicity of deoxycholate, a secondary bile acid. *Arch. Toxicol.* 85, 863-871.
- Caderni, G., Femia, A.P., Giannini, A., Favuzza, A., Luceri, C., Salvadori, M., Dolara, P., 2003. Identification of mucin-depleted foci in the unsectioned colon of azoxymethane-treated rats: correlation with carcinogenesis. *Cancer Res.*, 63, 2388-2392.
- Center, M.M., Jemal, A., Smith, R.A., Ward, E., 2009. Worldwide variations in colorectal cancer. *Cancer J. Clin.* 59, 366-378.

Degirolamo, C., Modica, S., Palasciano, G., Moschetta, A., 2011. Bile acids and colon cancer: Solving the puzzle with nuclear receptors. *Trends Mol. Med.* 17, 564-572.

Eyssen, H., De Pauw, G., Stragier, J., Verhulst, A., 1983. Cooperative formation of ω -muricholic acid by intestinal microorganisms. *Appl. Environ. Microbiol.* 45, 141-147.

Femia, A.P., Dolara, P., Caderni, G., 2004. Mucin-depleted foci (MDF) in the colon of rats treated with azoxymethane (AOM) are useful marker for colon carcinogenesis. *Carcinogenesis* 25, 277-281.

Hagio, M., Matsumoto, M., Fukushima, M., Hara, H., Ishizuka S., 2009. Improved analysis of bile acids in tissues and intestinal contents of rats using LC/ESI-MS, *J. Lipid Res.* 50, 173-180.

Houten, S.M., Watanabe, M., Auwerx, J., 2006. Endocrine functions of bile acids, *EMBO J.* 25, 1419-1425.

Ijiri, .K., Potten, C.S., 1983. Response of intestinal cells of differing topographical and hierarchical status to ten cytotoxic drugs and five sources of radiation. *Br. J. Cancer* 47, 175-185.

Ishizuka, S., Takeuchi, A., Hagio, M., Mohara, M., Sakai, H., Yamada, K., 2003. Relationship between formation of aberrant crypts and acute responses of colonic epithelial cells to genotoxic treatment among inbred rat strains. *Cancer Lett.* 196, 135-141.

Ishizuka, S., Shiwaku, M., Hagio, M., Suzuki, T., Hira, T., Hara, H., 2012. Glycochenodeoxycholic acid promotes proliferation of intestinal epithelia via reduction of cyclic AMP and increase in H2AX phosphorylation after exposure to γ -rays. *Biomed. Res.* 33, 159-165.

- Islam, K.B.M.S., Fukiya, S., Hagio, M., Fujii, N., Ishizuka, S., Ooka, T., Ogura, Y., Hayashi, T., Yokota, A., 2011 Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology* 141, 1773-1781.
- Kerr, J.F., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26, 239-257.
- Katsuma, S., Hirasawa, A., Tsujimoto, G., 2005. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem. Biophys. Res. Commun.* 329. 386-390.
- Lin, C.X., Umemoto, A., Seraj, M.J., Mimura, S., Monden, Y., 2001. Effect of bile acids on formation of azoxymethane-induced aberrant crypt foci in colostomized F344 rat colon. *Cancer Lett.* 169, 121-126.
- Liu, R., Zhao, R., Zhou, X., Liang, X., Campbell, D.J., Zhang, X., Zhang, L., Shi, R., Wang, G., Pandak, W.M., Sirica, A.E., Hylemon, P.B., Zhou, H. 2014. Conjugated bile acids promote cholangiocarcinoma cell invasive growth via activation of sphingosine 1-phosphate receptor 2. *Hepatology* in press.
- Magnuson, B.A., Carr, I., Bird, R.P., 1993. Ability of aberrant crypt foci characteristics to predict colonic tumor incidence in rats fed cholic acid. *Cancer Res.* 53, 4499-4504.
- Maruyama, T., Tanaka, K., Suzuki, J., Miyoshi, H., Harada, N., Nakamura, T., Miyamoto, Y., Kanatani, A., Tamai, Y., 2006. Targeted disruption of G protein-coupled bile acid receptor 1 (Gpbar1/M-Bar) in mice. *J. Endocrinol.* 191, 197-205.
- Potten, C.S., 1990. A comprehensive study of the radiobiological response of the murine (BDF1) small intestine. *Int. J. Radiat. Biol.* 58, 925-973.

Ochiai, M., Hippo, Y., Izumiya, M., Watanabe M., Nakagama, H., 2014. Newly defined aberrant crypt foci as a marker for dysplasia in the rat colon. *Cancer Sci.*, 105, 943-950.

Raju, J., 2008. Azoxymethane-induced rat aberrant crypt foci: relevance in studying chemoprevention of colon cancer. *World J. Gastroenterol.* 14, 6632-6635.

Reddy, B.S., 1981. Diet and excretion of bile acids, *Cancer Res.* 41, 3766-3768.

Reeves, P.G., Nielsen, F.H., Fahey, G.C.Jr., 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* 123, 1939-1951.

Sacquet, E.C., Raibaud, P.M., Mejean, C., Riottot, M.J., Leprince, C., Leglise, P.C., 1979. Bacterial formation of ω -muricholic acid in rats. *Appl. Environ. Microbiol.* 37, 1127-1131.

Sato, H., Macchiarulo, A., Thomas, C., Gioiello, A., Une, M., Hofmann, A.F., Saladin, R., Schoonjans, K., Pellicciari, R., Auwerx, J., 2008 Novel potent and selective bile acid derivatives as TGR5 agonists: biological screening, structure-activity relationships, and molecular modeling studies. *J. Med. Chem.* 51, 1831-1841.

Schlam, S.W., Larusso, N.F., Hofmann, A.F., Hoffman, N.E., van Berge-Henegouwen, G.P., Korman, M.G., 1978. Diurnal serum levels of primary conjugated bile acids. *Gut* 19, 1006-1014.

Sinicrope, F.A., Foster, N.R., Sargent, D.J., O'Connell, M.J., Rankin, C., Obesity is an independent prognostic variable in colon cancer survivors. *Clin. Cancer Res.* 15, 1884-1893.

Studer, E., Zhou, X., Zhao, R., Wang, Y., Takabe, K., Nagahashi, M., Pandak, W.M., Dent, P., Spiegel, S., Shi, R., Xu, W., Liu, X., Bohdan, P., Zhang, L., Zhou, H.,

- Hylemon, P.B., 2012. Conjugated bile acids activate the sphingosine-1-phosphate receptor 2 in primary rodent hepatocytes. *Hepatology* 55, 267-276.
- Takayama, T., Miyanishi, K., Hayashi, T., Sato, Y., Niitsu, Y., 2006. Colorectal cancer: genetics of development and metastasis. *J. Gastroenterol.* 41, 185-192.
- Walther, A., Johnstone, E., Swanton, C., Midgley, R., Tomlinson, I., Kerr, D., Genetic prognostic and predictive markers in colorectal cancer. *Nat. Rev. Cancer* 9, 489-499.
- Wu T, Bound, M.J., Standfield, S.D., Gedulin, B., Jones, K.L., Horowitz, M., Rayner, C.K., 2013. Effects of rectal administration of taurocholic acid on glucagon-like peptide-1 and peptide YY secretion in healthy humans. *Diabetes Obes. Metab.* 15, 474-477.
- Yang, J.I., Yoon, J.H., Myung, S.J., Gwak, G.Y., Kim, W., Chung, G.E., Lee, S.H., Lee, S.M., Kim, C.Y., Lee, H.S., 2007. Bile acid-induced TGR5-dependent c-Jun-N terminal kinase activation leads to enhanced caspase 8 activation in hepatocytes. *Biochem. Biophys. Res. Commun.* 361, 156-161.

Figure legends

Fig. 1. Epithelial status in the rat colon 4 days after γ -ray exposure. A: number of crypts per colon section. Two-way ANOVA *P*-values are 0.0013 for diet (D), <0.0001 for radiation dose (R), and 0.2338 for D x R. B: number of epithelial mitoses per colon section. Two-way ANOVA *P*-values are <0.0001 for diet (D), 0.0019 for radiation dose (R), and 0.2871 for D x R. The open and filled symbols are the values from rats fed a control or CA-supplemented diets, respectively. The data are expressed as the means $\pm$ SEM. **P* < 0.05 vs. control at the same dose.

Fig. 2. Epithelial kinetics of the rat colon after exposure to γ -rays. A: percent change in BrdU-labeled epithelial cells. Two-way ANOVA *P*-values are 0.0001 for diet (D), <0.0001 for time (T), and 0.2220 for D x T. B: percent changes in mitotic epithelial cells. Two-way ANOVA *P*-values are 0.1338 for diet (D), <0.0001 for time (T), and 0.8177 for D x T. C: changes in the apoptotic index in the epithelial cells. Two-way ANOVA *P*-values are 0.0003 for diet (D), <0.0001 for time (T), and 0.0236 for D x T. The open and filled symbols are values from the rats fed the control or CA-supplemented diets, respectively. The data are expressed as the means $\pm$ SEM. **P* < 0.05 vs. control. D: comparison between the distribution of BrdU-incorporated cells in unirradiated rats (solid lines) and apoptotic index at 3 h after exposure to 4 Gy of γ -rays (broken lines) over the colon crypts. * Significant difference (*P* < 0.05) between the values of the BrdU-incorporated cell frequency and apoptotic index at each cell position (CP).

Fig. 3. Cecal extracts or sera of rats fed the control or CA-supplemented diet promoted IEC-6 cell proliferation. The cells were incubated for the indicated numbers of days with media containing the cecal extracts diluted with DMEM (Two-way ANOVA *P*-values are <0.0001 for diet (D), <0.0001 for time (T), and 0.0035 for D x T in the treatment with the cecal extracts) or rat sera (Two-way ANOVA *P*-values are <0.0001 for diet (D), <0.0001 for time (T), and 0.1382 for D x T in the treatment with the sera). The open and solid bars are the values from the rats fed the control or CA-supplemented diet, respectively. The data are expressed as the means $\pm$ SEM. **P* < 0.05 vs. control diet.

Fig. 4. Epithelial status in the rat colon 8 weeks after the exposure to γ -radiation (4 Gy).

A: counts of BrdU-incorporating epithelial cells per half-crypt section in the rat colon.

B: counts of whole epithelial cells per half-crypt section in the rat colon. The data are expressed as the means $\pm$ SEM. * $P < 0.05$ vs. control diet.

Highlights

1. Cholic acid (CA) diet (2 g/kg diet) promotes colonic epithelial proliferation.
2. No influence of the CA diet on the epithelial apoptosis induced by γ -irradiation.
3. Cecal extracts and sera from CA-fed rats promote IEC-6 cell proliferation.
4. The CA ingestion disturbs the BA distribution and profile.

Table 1
Diet composition

	Control	CA
	g/kg diet	
Sucrose ¹	599.5	597.5
Casein ²	250.0	250.0
Soybean oil ³	50.0	50.0
Cellulose ⁴	50.0	50.0
Mineral mixture ⁵	35.0	35.0
Vitamin mixture ⁶	10.0	10.0
L-cystine ⁷	3.0	3.0
Choline chrolide ⁸	2.5	2.5
Cholic acid ⁹	-	2.0

¹ Sucrose (Nippon Beet Sugar Mfg. Co., Ltd., Obihiro, Japan).

² NZMP Acid Casein (Fonterra, Ltd., Auckland, New Zealand).

³ Soybean oil (J-Oil Mills, Inc., Tokyo, Japan).

⁴ Ceolus (Asahi Kasei Chemicals Co., Ltd., Tokyo, Japan).

^{5, 6} Mineral and vitamin mixtures were prepared according to the AIN-93G formulation.

^{7, 8, 9} Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Table 2

Growth parameters, tissue weights, and bile flow

	Control	CA	
Growth parameters (g)			
Final body weight	94.3 ± 3.2	85.2 ± 4.0	*
Body weight gain	35.4 ± 0.9	26.2 ± 2.1	*
Total food intake	99.6 ± 4.4	84.9 ± 3.6	*
Tissue weight (g/100 g body weight)			
Liver	4.52 ± 0.14	4.49 ± 0.10	
Jejunum	0.85 ± 0.05	0.79 ± 0.06	
Ileum	0.52 ± 0.05	0.56 ± 0.03	
Cecum	0.32 ± 0.02	0.38 ± 0.02	
Colorectum	0.33 ± 0.01	0.36 ± 0.02	
Mesenteric adipose tissue	0.59 ± 0.03	0.42 ± 0.02	*
Perirenal with dorsal adipose tissue	0.46 ± 0.04	0.23 ± 0.04	*
Epididymal adipose tissue	0.56 ± 0.04	0.35 ± 0.04	*
Bile flow (ml/100 g body weight/h)	0.38 ± 0.03	0.62 ± 0.04	*

* Significant difference ($P < 0.05$, $n = 6$). Values are expressed as means with SEM.

Table 3
Bile acid concentration in dry feces

	Control	CA
	(μmol/g dry feces)	
Unconjugated		
CA	0.02 ± 0.01	15.87 ± 3.81 *
αMCA	0.05 ± 0.02	0.44 ± 0.05 *
βMCA	0.30 ± 0.06	1.00 ± 0.24 *
ωMCA	1.47 ± 0.59	0.57 ± 0.27
HDCA	0.04 ± 0.02	0.03 ± 0.01
UDCA	0.01 ± 0.01	0.07 ± 0.01 *
CDCA	< 0.01	0.04 ± 0.01 *
DCA	0.41 ± 0.23	12.16 ± 4.21 *
LCA	0.11 ± 0.04	0.06 ± 0.02
Taurine-conjugated		
CA	< 0.01	0.57 ± 0.23 *
αMCA	ND	0.03 ± 0.01 *
ωMCA	< 0.01	< 0.01
HDCA	ND	< 0.01
CDCA	ND	< 0.01
DCA	< 0.01	0.45 ± 0.20 *
LCA	ND	ND
Glycine-conjugated		
CA	ND	0.04 ± 0.02 *
HDCA	ND	ND
UDCA	ND	ND
CDCA	ND	ND
DCA	ND	0.03 ± 0.01 *
LCA	ND	ND
TBA	2.43 ± 0.95	31.35 ± 2.98 *
BA excretion (μmol/day)	1.22 ± 0.36	15.00 ± 2.74 *

* Significant difference ($P < 0.05$, $n = 6$). TBA is showed as the sum of detected BA using UPLC-MS. BA excretion is calculated from TBA amount in feces and weight of feces. Values are expressed as means with SEM.

Table 4
Bile acid concentration in serum

	Control	CA
	(nmol/ml)	
Unconjugated		
CA	0.16 ± 0.06	3.79 ± 1.06 *
αM CA	0.04 ± 0.02	0.09 ± 0.03
βMCA	0.04 ± 0.01	0.16 ± 0.03 *
ωMCA	0.09 ± 0.03	0.02 ± 0.01
HDCA	< 0.01	< 0.01
UDCA	< 0.01	0.04 ± 0.01 *
CDCA	0.07 ± 0.04	0.08 ± 0.02
DCA	0.02 ± 0.01	1.52 ± 0.80
LCA	ND	ND
Taurine-conjugated		
CA	0.25 ± 0.11	1.96 ± 0.64 *
αMCA	0.02 ± 0.01	0.02 ± 0.01
ωMCA	0.02 ± 0.01	< 0.01
HDCA	0.02 ± 0.01	< 0.01
CDCA	0.01 ± 0.01	< 0.01
DCA	0.01 ± 0.01	0.31 ± 0.12 *
LCA	ND	ND
Glycine-conjugated		
CA	0.01 ± 0.01	0.67 ± 0.21 *
HDCA	< 0.01	< 0.01
UDCA	< 0.01	< 0.01
CDCA	< 0.01	< 0.01
DCA	< 0.01	0.09 ± 0.04 *
LCA	ND	ND
TBA	0.79 ± 0.22	8.78 ± 1.12 *

* Significant difference ($P < 0.05$, $n = 6$). TBA is showed as the sum of detected BA using UPLC-MS. Values are expressed as means with SEM.

Figure 1

Figure 2

Figure 3

Figure 4