〈Brief Note〉

Effects of horseradish leaf extracts on young mice fed an excessive cholic acid supplemented diet: a preliminary study

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Summary Horseradish is mainly cultivated for its root, and its leaves are rarely eaten. Cholic acid (CA) is a primary bile acid (BA) synthesized from cholesterol in the liver and plays a central role in the digestion and absorption of dietary fat. Excess amounts of CA are metabolized to secondary BAs which have inflammatory activities in the large intestine. In this study, male ICR mice were fed a diet supplemented with 1.2% (w/w) CA, and/or 0.3% horseradish leaf extracts (HLE). CA inhibited weight gain in the mice, whereas HLE blocked these effects of CA. Moreover, HLE blocked the increase in the weight of the large intestine, but failed to inhibit that of the liver in the mice. Our results show that HLE inhibits the negative impact induced by CA, and the effects of HLE on the body and intestinal weight of mice are independent of those on the liver.

Key words: horseradish leaf extracts, mice, cholic acid

1. Introduction

Horseradish (*Armoracia rusticana*) is a perennial crop belonging to the Brassicaceae family, and its roots are widely used for culinary purposes. Horseradish root has anti-inflammatory and antioxidative activities; it can reportedly suppress TNF-α and IL-6 release in J774A.1 macrophages treated with lipopolysaccharide (LPS)[1]. Additionally, it is effective in the treatment of acute sinusitis, bronchitis, and urinary bladder infections[2] and inhibits cell proliferation in colon and lung cancer cells[3]. Wasabi is a pungent spice belonging to the Brassicaceae family and the beneficial effects of its leaves and roots have been studied[4]. However, horseradish leaves are often discarded because they are not considered edible. Therefore, the beneficial effects of horseradish leaves are poorly documented[5,6].

Cholic acid (CA) is a major primary bile acid (BA) synthesized from cholesterol in the liver. CA is conjugated with taurine or glycine and plays an essential role in digestion as it emulsifies and solubilizes fat in the small intestine. Most primary bile

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acids are reabsorbed in the distal ileum and recycled to the liver. However, small amounts of primary bile acids transit to the large intestine where taurine or glycine become unconjugated from the primary BA. Excessively secreted CA is metabolized to secondary BAs such as deoxycholic acid (DCA) in the colon[7-9]. DCA is a risk factor for inflammation and cancer of the colon and inhibits macrophage activation[9,10]. Studies have shown that the proliferation of colonic mucosal cells was stimulated by CA[11] and that CA is also a promoter of cancer of the large intestine[12]. In this study, we explored the therapeutic effects of horseradish leaf extracts (HLE) on the negative impact induced by CA in mice.

2. Materials and Methods

Chemicals and preparation of HLE

Cholic acid was purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). HLE was provided by Amino Up Co., Ltd. (Sapporo, Japan).

HLE was prepared as follows: (1) Dried horseradish leaves were heated in water at 70°C for 120 min; (2) hot water extract of horseradish leaves and the resulting extract were centrifuged at 5000×g for 10 min; (3) the supernatant was collected and filtered; (4) dilution agent (dextrin) was added to the supernatant; (5) the supernatant was heated at 80°C for 30 min and freeze-dried for later use. This extraction was performed by Amino Up Co., Ltd. The solid capture rate of HEL is about 24% and the same quantity of pinedex was added as vehicle.

Animals and experimental design

Male ICR mice (5 weeks old) were obtained from Kiwa Laboratory Animals Co., Ltd. (Kimino, Japan). Mice were maintained at the following conditions: temperature of 24 ±1°C, relative humidity of 55 ± 5%, 12 h light/dark cycle, and acclimatization for one week before the start of experiments with free access to water and normal chow powdered food (MF; Oriental Yeast Co., Ltd, Tokyo, Japan). The mice were classified randomly into four groups (n=4-5 for each group): (1) normal diet as control group, (2) 1.2% (w/w) CA in normal chow powdered food, (3) 0.3% HLE (w/w) in normal chow powdered food, (4) and 1.2% CA+0.3% HLE in normal chow powdered food[13], with inclusion of unpublished data. For CA mice, the normal diet was replaced with a diet supplemented with CA at 8 days after the start of the experiment. HLE mice were fed a diet containing HLE for the entire experimental period, and CA was supplemented with HLE after 8 days in the CA plus HLE mouse group. Food consumption and body weight were measured 2-3 times a week, and body weight gain was calculated. The mice were euthanized intraperitoneally using a mixture of three types of anesthetic agents (medetomidine, midazolam, and butorphanol) and liver were removed and weighed. The large intestine was opened, and fecal contents were rinsed with phosphate-buffered saline and weighted. Weights of liver and the large intestine were calculated as per 100 g of the body weight and per 100 g of the body weight/ 1 cm of length, respectively. Animal experiments were approved by the Institutional Animal Care and Use Committee (permission number: kyo31-2) and performed according to the Tokyo Healthcare University Animal Experimentation Regulations.

Statistical Analysis

Results are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed by Student's t-test and a p value of less than 0.05 was considered as statistically significant.

3. Results

Effects of HLE on body weight in mice fed a diet supplemented with CA

Changes in the body weight gain of mice are shown in Fig. 1. The control mice had gained 6.64 ± 0.52 g of body weight on day 12. However, the CA-group mice showed 0.14 ± 0.65 g of weight gain which was reduced significantly compared to the control (2.1% of the control, $p<0.0001$). HLE significantly impaired the reduction of body weight compared to CA-supplementation (31.9% of the

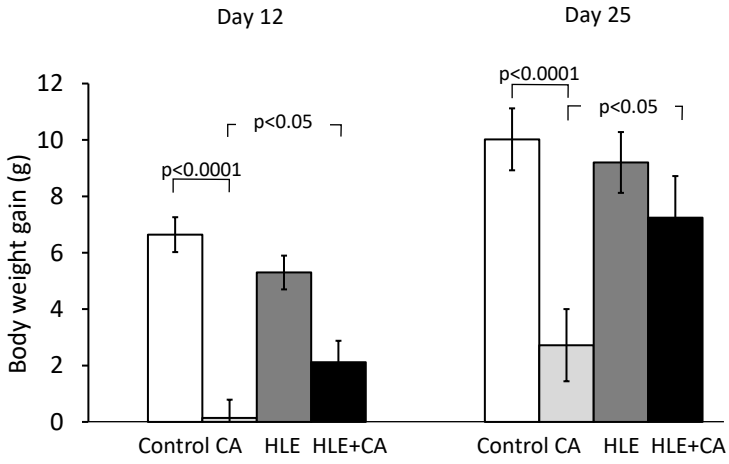

Fig. 1. Effects of HLE on weight loss in mice fed a CA supplemented diet. Male ICR mice (5 weeks old, 29.7—36.5 g) were randomly divided into control group (Control), 0.3% (w/w) CA (CA), 1.2% HLE (HLE), and 0.3% CA + 1.2% HLE (CA + HLE). Data are expressed as mean ± standard error (SE).

control, 2.12 ± 0.76 g, $p<0.05$). Further, HLE inhibited the reduction in body weight by CA by day 25 (25.1% of the control, CA: 2.72 ± 1.28 g and 66.8% of the control, CA+HLE: 7.24 ± 1.48 g, $p<0.05$). A diet with HLE alone did not affect the body weight on days 12 or 25. The food intake per day was 4.4-5.6 g in all groups.

Effects of HLE on the large intestine in mice fed a CA supplemented diet

To study the effects of CA and/or HLE on colonic mucosa, we determined the weight of the large intestine (Fig. 2). CA significantly increased the weight of the large intestine (control: 0.077 ± 0.003, 147% of the control, CA: 0.114 ± 0.011 g weight/body weight (100 g)/length, $p<0.05$). HLE blocked the increase in weight of the large intestine in CA mice (111% of the control, HLE+ CA: 0.086 ± 0.003 g weight/body weight (100 g)/length, $p<0.05$). HLE administered alone caused a significant reduction

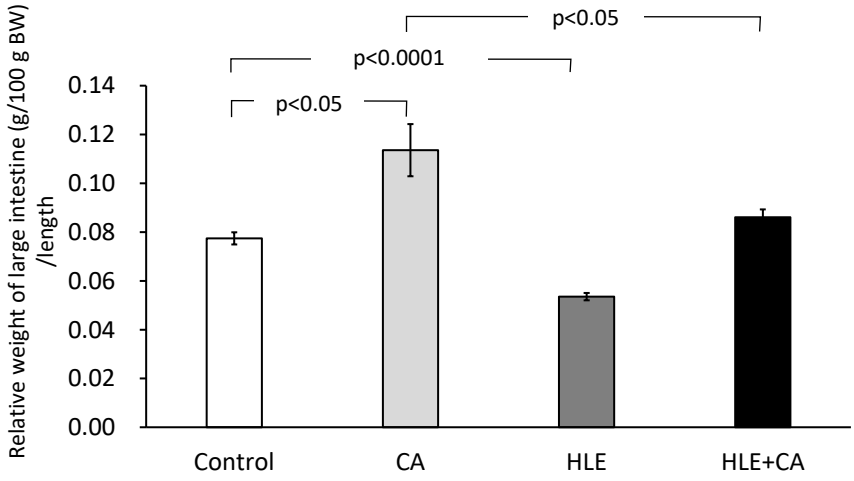

Fig. 2. Effects of HLE on the weight of the large intestine in mice fed a CA supplemented diet. The relative weight of the large intestine is expressed as the weight/100 g BW/length. Data are expressed as means ± SE. Control: normal diet, CA: diet supplemented with CA, HLE: diet with HLE, HLE + CA: supplemented with HLE + CA.

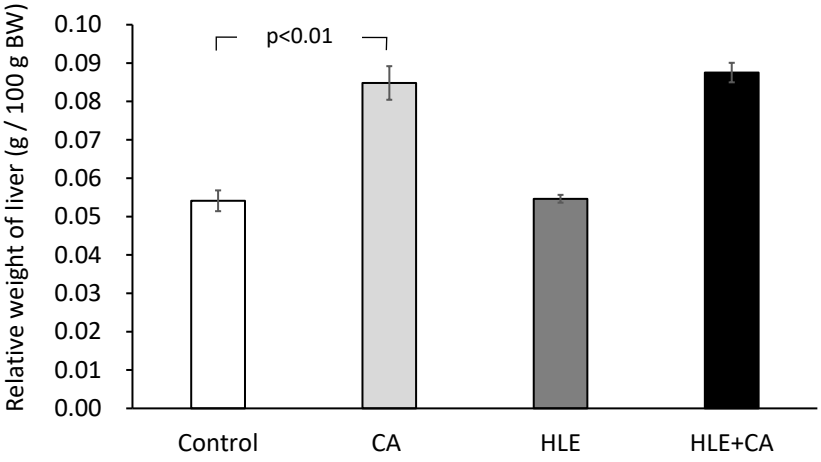

Fig. 3. Effects of HLE on the liver weight in mice fed a CA supplemented diet. The relative weight of the liver is expressed as the weight/100 g BW. Data are expressed as the mean ± SE. Control: normal diet, CA: diet supplemented with CA, HLE: diet with HLE, HLE + CA: supplemented with HLE + CA

in the large intestine (69% of the control, HLE: 0.054 ± 0.002 g weight/body weight (100 g)/length, $p<0.0001$).

Effects of HLE on the liver weight in mice fed a CA supplemented diet

Next, we evaluated the changes in liver weight (Fig. 3). CA supplementation induced a significant increase in the relative weight of the liver (control: 0.052 ± 0.002 g weight/body weight (100 g), 163% of the control, CA: 0.085 ± 0.004 g, $p < 0.001$). However, changes in the relative weight of the liver remained unaffected in mice fed a diet supplemented with CA + HLE (168% of the control, 0.088 ± 0.003 g weight/body weight (100 g)). Administration of HLE alone also did not affect the liver weight (105% of the control).

4. Discussion

Horseradish is a pungent spice that belongs to the Cruciferae family and its root extracts have several bioactivities including anti-inflammatory or anti-oxidative effects[2,3]. However, there are few studies of horseradish leaf extracts in experimental animals or humans [5,6]. In this study, we investigated the effects of HLE on mice fed a CA supplemented diet, and found that HLE blocked the inhibitory effects of CA on the body weight gain without affecting the diet intake. We showed that the increase in colon weights was recovered by HLE in mice fed a CA-containing diet. However, HLE failed to affect the liver weight in CA mice.

Feeding a diet supplemented with CA reportedly increases the levels of fecal and colonic DCA[14] which is known to induce oxidative stress, cytotoxicity, and apoptosis in experimental animals[15-17]. Oral administration of DCA has been also shown to enhance indomethacin-induced enteropathy in rats[18]. The mechanisms mediating the inhibitory effects of CA on the gain in bodyweight are unclear. However, the detrimental effects of CA may occur through oxidative stress-induced signaling. Glucosinolates are secondary plant metabolites abundantly found in Brassica plants and the amount in horseradish leaves and roots were almost equal[19]. Sinigrin is a glucosinolate found in horseradish leaves[19] and reportedly has anti-inflammatory and antioxidant activities in rats[20]. Induction of anti-inflammatory and/or antioxidant activity by HLE may be one of the mechanisms associated with the inhibition of the negative effects of CA on the body weight of mice. Further studies are required for the clarification of the detailed mechanism.

In this study, CA increased the relative weights of the intestine and liver in mice. Epidemiologic studies have shown that a high-fat diet (HFD)

induces elevated levels of fecal secondary bile acids, mainly DCA[9,21]. Furthermore, HFD induced intestinal hyperpermeability, colonic inflammation, and hepatomegaly in rodents[22-25]. We showed that HLE blocked the decrease in body weight and the increase in intestine weight by CA administration. Anti-inflammatory effect of HLE on the intestine may be, at least in part, a mechanism for inhibition of the negative impact such as loss body weight induced by CA. On the other hand, HLE failed to inhibit liver weight gain in mice fed a diet supplemented with CA, and a diet with HLE alone reduced the intestine weight but not the body or the liver weight, suggesting that the effects of HLE on the body weight and the liver occur via a different mechanism compared to the intestine. Recent studies have shown that BA are signaling molecules involved in several physiological functions via the activation of nuclear receptors[26,27]. These receptors have a selective affinity to different types of BAs and show different expression patterns, resulting in different roles for BAs. Different effects of CA on the organs or tissues may occur through signal transduction by various secondary BAs derived from CA.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

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References

1. Marzocco S, Calabrone L, Adesso S, Larocca M, Franceschelli S, Autone G, Martelli G and Rossano R: Anti-inflammatory activity of horseradish (Armoracia rusticana) root extracts in LPS-stimulated macrophages. Food Dunct, 6:3778-3788, 2015.
2. Goos K-H, Albrecht U and Schneider B: Efficacy and safety profile of a herbal drug containing nasturtium herb and horseradish root in acute sinusitis, acute bronchitis and acute urinary tract infection in comparison with other treatments in the daily practice / results of a prospective cohort study. Arzbeimittelforschung, 62:249-257, 2013.
3. Weil MJ, Zhang Y and Nair MG: Tumor cell proliferation and cyclooxygenase inhibitory constituents in horseradish (armoracia rusticana) and wasabi (wasabia japonica). J Agri Food Chem, 5:1440-1444, 2005.
4. Kinae N, Masuda H, Shin IS, Furugori M and Shimoi K: Functional properties of wasabi and horseradish. Biofactors, 13:265-269, 2000.
5. Walters SA: Horseradish: a neglected and underutilized plant species for improving human health. Horticulturae, 7:167, 2021.
6. Majewska A, Batasinska B and Dqbrowska B: Antioxidant properties of leaf and root extract and oil from different types of horseradish (Armoracia rusticana Gaertn). PSHS, 16:15-22, 2004.
7. Begley M, Gahan CGM and Hill C: The interaction between bacteria and bile. FEMS Microbiology Reviews, 29:625-651, 2005.
8. Fukui H: Leaky gut and gut-liver axis in liver cirrhosis: clinical studies update. Gut and Liver, 15:666-676, 2021.
9. Zeng H, Umar S, Rust B, Lazarova D and Bordonaro M: Secondary bile acids and short chain fatty acids in the colon: a focus on colonic microbiome, cell proliferation, inflammation, and cancer. Int J Mol Sci, 20:1214, 2019.
10. Wang L, Gong Z, Zhang X, Zhu F, Kiu Y, Jin C, Du X, Xu C, Chen Y, Cai W, Tian C and Wu J: Gut microbial bile acid metabolite skews macrophage polarization and contributes to high-fat diet-induced colonic inflammation. Gut Microbes, 12:e1819155-e1819175, 2020.
11. Awad AB, Hernandez AY, Fink CS and Mendel SL: Effects of dietary phytosterols on cell proliferation and protein kinase C activity in rat colonic mucosa. Nutr Cancer, 27:210-215, 1997.
12. Sun Y and Li Y: Induction of beta-glucuronidase activity during dimethylhydrazine carcinogenesis and additive effects of cholic acid indole. Cancer Lett, 39:69-76, 1988.
13. Islam KBMS, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, Ogura Y, Hayashi T and Yokota A: Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology, 141:1773-1781, 2011.
14. Batta AK, Salen G, Holubec H, Brastius TA, Alberts

D and Earnest DL: Enrichment of the more hydrophilic bile acid ursodeoxycholic acid un the fecal water-soluble fraction after feeding to rats with colon polyps. Cancer Res, 58:1684-1687, 1998.

15. Yamada S, Kawaguchi H, Yamada T, Guo X, Matsuo K, Hamada T, Miura N Tasaki T and Tanimoto A: Cholic acid enhances visceral adiposity, atherosclerosis and nonalcoholic fatty liver disease in microminipigs. J Atheroscier Thromb, 24:1150-1166, 2017.
16. Vallim TQA, Tarling EJ and Edwards PA: Pleiotropic roles of bile acids in metabolism. Cell Metab, 17:657-659, 2013.
17. Ferreira DMS, Afonso MB, Rodrigus PM, Simao AL, Pereira DM, Borralho PM, Rodrigues CMP and Castro RE: c-Jun n-terminal kinase 1/c-jun activation of the p53/microRNA 34a/sirtuin 1 pathway contributes to apoptosis induced by deoxycholic acid in rat liver. Mol Cell Biol, 34:1100-1120, 2014.
18. Shibuya N, Higashiyama M, Akita Y, Shirakabe K, Ito S, Nishii S, Mizoguchi A, Inaba K, Tanemoto R, Sugihara N, Hanawa Y, Wada A, Horiguchi K, Yoshikawa K, Kurihara C, Okada Y, Watanabe C, Komoto S, Tomota K, Saruta M and Hokari R: Deoxycholic acid enhancement of lymphocyte migration through direct interaction with the intestinal vascular endothelium. J Gastroentrol Hepatol, 36:2523-2530, 2021.
19. Popovic M, Maravic A, Culic VC, Dulovic A, Burcul F and Blazevic I: Biological effects of glucosinolate degradation products from horseradish: a horse that wins the race. Biomolecules, 10:343-360, 2020.
20. Mazumder A, Dwivedi A and Du Plessis J: Sinigrin and its therapeutic benefits. Molecules, 21:416-432, 2016.
21. Ajouz H, Mukherji D and Shamseddine A: Secondary bile acids: an underrecognized cause of large intestine cancer. World J Surg Oncol, 12:164, 2014.
22. Murakami Y, Tanabe S and Suzuki T: High-fat diet-induced intestinal hyperpermeability is associated with increased bile acids in the large intestine of mice. J Food Sci, 81:H216-H222, 2016.
23. Zhao S, Gong Z, Zhou J, Tian C, Gao Y, Xu C, Chen Y, Cai W and Wu J: Deoxycholic acid triggers NLRP3 inflammasome activation and aggravates DSS-induced colitis in mice. Front Immunol, 7:1-11, 2016.
24. Repa JJ, Lund EG, Horton JD, Leitersdorf E, Russell DW, Dietschy JM and Turley SD: Disruption of the sterol 27-hydroxylase gene in mice results in hepatomegaly and hypertriglyceridemia. J Biol Chem, 275:39685-39692, 2000.
25. Devkota S and Chang EB: Interactions between diet, bile acid metabolism, gut microbiota,and inflammatory bowel diseases. Dig Dis, 33:351-356, 2015.
26. Basptissart M, Vega A, Maqdasy S, Caira F, Baron S, Lobaccaro JMA and Volle DH: Bile acids: from digestion to cancers. Biochemie, 95:504-517, 2013.
27. Martinot E, Sedes L, Baptissart M, Lobaccaro JM, Caira F, Beaudoin C and Volle DH: Bile acids and their receptors. Mol Aspects Med, 56:2-6, 2017.