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Intestinal Bacterial β-Glucuronidase Activity of Patients with Colon Cancer

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The fecal β-glucuronidase activity of patients with colon cancer and healthy controls were measured to determine the relationship between the fluctuation of intestinal bacterial β-glucuronidase and colon cancer. The fecal β-glucuronidase activity of patients with colon cancer was 1.7 times higher than that of the healthy controls. However, when these fecal specimens were sonicated, the enzyme activity of patients with colon cancer was 12.1 times higher than that of the healthy controls. The fecal β-glucuronidase activity of human intestinal bacteria was drastically induced by its substrate or the bile secreted after a subcutaneous injection of 1,2-dimethylhydrazine (DMH) and benzo[a]pyrene into rats. DMH- and benzo[a]pyrene-treated biles induced β-glucuronidase activity in the human intestinal microflora by approximately 1.5- and 2.3-fold, respectively. They also induced β-glucuronidase in *E. coli* HGU-3, which is a β-glucuronidase-producing bacterium from the human intestine. D-saccharic acid 1,4-lactone similarly inhibited fecal β-glucuronidase in several patients with colon cancer in addition to the healthy controls. This suggests that potent β-glucuronidase activity is a prime factor in the etiology of colon cancer.

Key words: Colon cancer, β-Glucuronidase, Intestinal bacteria, 1,2-Dimethylhydrazine

INTRODUCTION

Epidemiological studies suggest that dietary factors, such as those high in animal fat and protein, are prime factors in the etiology of colon cancer (Goldin and Gorbach, 1976: Weisburger, 1977). The following hypothesis has been suggested by many researchers (Hill, 1975: Reddy *et al* 1975: Wynder and Reddy, 1975). Dietary fat changes the bile acid and cholesterol metabolites both quantitatively and qualitatively, as well as the concentration and metabolic activity of bacteria in the colon, which may produce carcinogens or carcinogenic compounds from the bile acid and cholesterol metabolites. Intestinal bacteria may play an important role in liberating the active key intermediates with chemical carcinogens inducing colon tumors in experimental animals (Gorbach and Gorbach, 1976). It is thought that 1,2-dimethylhydrazine (DMH) injected into a rat may be conjugated with glucuronic acid immediately in the liver and be secreted via the bile duct to the intestine. The glucuronic acid conjugate would be hydrolyzed by bacterial β-glucuronidase into the free compound, producing a relatively highly localized concentration of this compound in the colonic mucosa. The active carcinogen causes colonic cancer (Fiala, 1975 and 1977). However, the relationship between the intestinal bacterial β-glucuronidase activity and colon cancer patients was not investigated. This study examined the bacterial enzymes, β-glucuronidase, which are induced in DMH colon cancer model animals, and its inhibitors that prevent colonic cancer in these model animals (Kim *et al.*, 1995).

Therefore, the intestinal bacterial β-glucuronidase activity of colon cancer patients was compared with that of healthy persons and the factors inducing β-glucuronidase activity were investigated.

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MATERIALS AND METHODS

Materials

p-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-β-D-glucuronide, 1,2-dimethyl hydrazine (DMH), benzo[a]

pyrene and D-saccharic acid 1,4-lactone were purchased from the Sigma Chem. Co. (U.S.A.). The general anaerobic medium (GAM) was purchased from the Nissui Pharm. Co., Ltd (Japan).

Subjects

Thirteen patients (age range 40-60 years; 8 male, 5 female) who were diagnosed with colon cancer in Kangnam Sungshim Medical Center and Kyunghee Medical Center, and 5 healthy Korean people (age range, 20-60 years; 3 males, 2 females) were enrolled in this study. The exclusion criteria included current medication, particularly regular or current use of antibiotics.

Specimens preparation

Fecal specimens were collected in plastic cups 9 h after fasting and the wet weight and bacterial enzyme activities were determined within 24 h. These specimens were carefully mixed with a spatula and suspended in cooled tubes containing 20-fold saline. Samples of the fecal suspension were prepared as follows.

Preparation 1 - The fecal suspension was centrifuged at 500 rpm for 5 min. The supernatant was used to assay the enzyme activity.

Preparation 2 - The fecal suspension was centrifuged at 500 rpm for 5 min, sonicated for 4 min (Ultrasonic processor, HEAT system Inc.), centrifuged at 10000 rpm for 20 min, and used for the enzyme activity assay.

Enzyme activity assay

β-Glucosidase and β-glucuronidase activities were assayed as follows (Kim *et al.*, 1994 and 1995): the reaction mixture (total volume of 0.5 ml) contained 0.2 ml of 2 mM *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase (or 1 mM *p*-nitrophenyl-β-D-glucuronidase for β-glucuronidase), 0.2 ml of a 0.1 M phosphate buffer, pH 7.0, and 0.1 ml of the fecal suspension (wet weight, 4 mg). The assay mixture was incubated at 37°C for 15 min. The reaction was quenched by adding 0.5 ml of 0.5 N-NaOH. The mixture was then centrifuged at 3000 rpm for 10min and the absorbance was measured at 405 nm (UV-vis spectrophotometer, Shimadzu UV-1201).

Animals

Male Sprague-Dawley rats (180-250g) were purchased from the Daehan Animal Co. (Korea). They were divided randomly into 3 groups of 3 animals (the normal group, the DMH-treated group and the benzo[a]pyrene-treated group, and were housed in steel wire cages. The animals had free access to a Samyang diet and water for two weeks.

To collect the bile, saline, DMH (20 mg/kg) and benzo *[a]pyrene* (10 mg/kg) were injected subcutaneously into the rats. Thirty minutes after the injection, the rats were anesthetized with urethane, their abdomens were opened and canulated to the bile duct. The bile was collected over a 6 h period.

Culture of intestinal bacteria in the medium containing bile

One gram of fresh feces from healthy men (20-30 years of age) was aseptically collected and immediately suspended in 9 ml of GAM broth. The suspended sample was inoculated into the GAM containing bile cultured for 20 h at 37°C, centrifuged at 5000 rpm for 20 min and suspended in 10 ml of 25 mM phosphate buffer at pH 7.0. It was then used as the enzyme solution. All procedures were carried out at 4°C.

Statistics

The significance of the differences between the groups was calculated using a Students *t*-test, Chi square test and an ANOVA test

RESULTS AND DISCUSSION

To understand the relationship between the fluctuations in the intestinal bacterial β-glucuronidase level and colon cancer, the fecal β-glucuronidase activities of the patients with colon cancer and healthy persons were measured (Fig. 1). Eighteen people (13 patients with colon cancer and 5 healthy controls) were enrolled in this study. There was no gender and age bias in these two groups as determined by a Chi-square test and ANOVA,

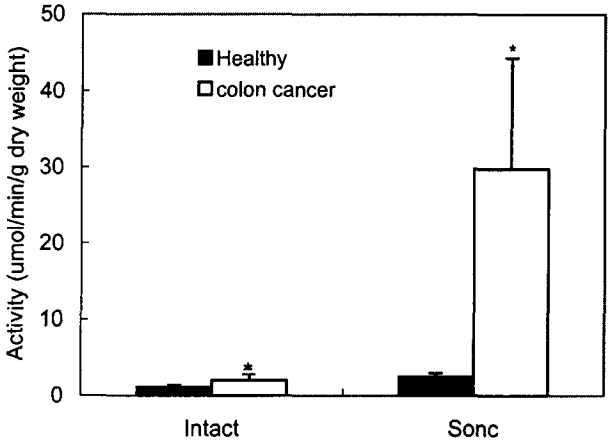

Fig. 1. The fecal β-glucuronidase activities of the patients with colon cancer, and healthy persons. Black bar, healthy persons; white bar, patients with colon cancer; intact, enzyme solution prepared according to the preparation 1 method in MATERIALS AND METHODS; sonic, enzyme solution prepared according to the preparation 2 method in MATERIALS AND METHODS. *Statistically significance when compared with the controls ($p<0.05$).

respectively. When the β-glucuronidase activities were measured by the Preparation 1 method, the fecal β-glucuronidase activity of patients with colon cancer was 1.7 times higher than that of the healthy controls. When these fecal specimens were sonicated, the activity of healthy persons increased 1.8-fold. The fecal β-glucuronidase activity of patients with colon cancer drastically increased 12.1-fold. This suggests that β-glucuronidase accumulates more in the intestinal bacteria of patients with colon cancer than in bacteria of healthy persons, which can potently hydrolyze the glucuronic acid conjugates. Therefore, to determine the β-glucuronidase inducing factor, DMH and benzo[a]pyrene was injected subcutaneously into rats and the bile collected, human intestinal bacteria were cultured in the GAM broth containing these biles, and β-glucuronidase activities was measured (Fig. 2). The DMH- and benzo[a]pyrene-treated biles induced β-glucuronidase 1.5 times and 2.3 times higher than the controls, respectively. However, they did not induce β-glucosidase. They also induced the β-glucuronidase in *E. coli* HGU-3, which was reported to be a β-glucuronidase-producing bacterium from the human intestinal bacteria in a previous study (Kim *et al.*, 1994), 1.2 1.6-fold. Therefore, the effect of PNGU, a synthetic β-glucuronidase substrate, on β-glucuronidase activities in human intestinal bacteria was determined (Fig. 3). This compound at a 0.1 mM concentration also induced the β-glucuronidase activity 4.6-fold. These results suggest that bile containing the glucuronic acid conjugates of the xenobiotics and endogenous compounds could induce the β-glucuronidase in fecal bacteria and promote colon cancer. These results are supported by Fiala (1975 and 1977), who reported that DMH- and benzo[a]pyrene subcutaneouly administered were detoxified in the liver by the conjugation of glucuronic acid or sulfuric acid and then secreted into intestine via the bile duct. To investigate the properties of β-glucuronidase, the inhibitory activity of D-saccharic acid 1,4-lactone, a well-known inhibitor, on the fecal β-glucuronidase activities of patients with colon cancer and healthy controls was measured (Fig. 4). These fecal β-glucuronidases were inhibited by D-saccharic acid 1,4-lactone. D-saccharic acid 1,4-lactone similarly inhibited fecal β-glucuronidase in some patients with colon cancer and healthy controls. Therefore, the difference between the β-glucuronidase(s) activity in healthy persons and patients with colon cancer is not thought to be significant.

In addition, Thorton *et al.* (1981) reported that a high colonic pH could promote colon cancer. We also reported that the β-glucuronidase activity in fecal bacteria could be induced 5-fold in an alkaline pH, compared to that in pH 6 (Kim *et al.*, 1994).

In conclusion, the potent β-glucuronidase activity caused by the glucuronic acid conjugates from xenobiotics and endogenous compounds is a prime factor in the etiology

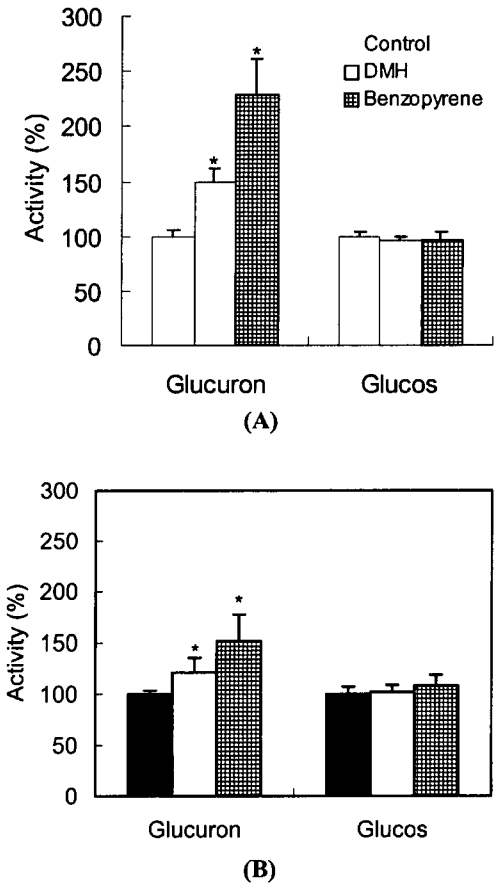

Fig. 2. The effects of DMH- and benzo[a]pyrene-treated biles on the induction of β-glucuronidase activities of human intestinal bacteria. (A), Human intestinal microflora; (B), E. coli HGU-3. Control, added saline-treated bile into the media; DMH, added dimethylhydrazine-treated bile into the media; Benzopyrene, added benzo[a]pyrene-treated bile into the media; Glucuron, β-glucuronidase; Glucos, β-glucosidase. *Statistically significance compared with the control group ($p < 0.05$).

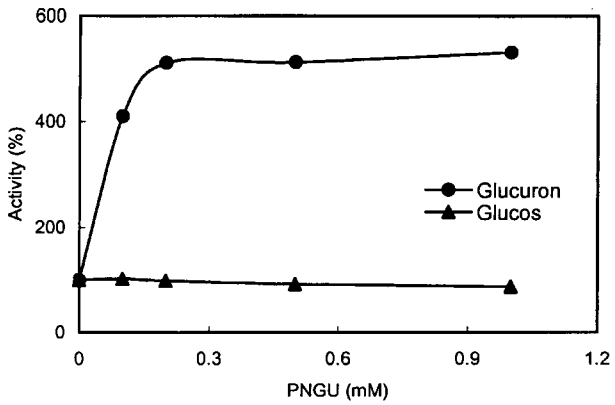

Fig. 3. The effect of PNGU on the induction of β-glucuronidase activities of human intestinal bacteria. Glucuron, β-glucuronidase; Glucos, β-glucosidase.

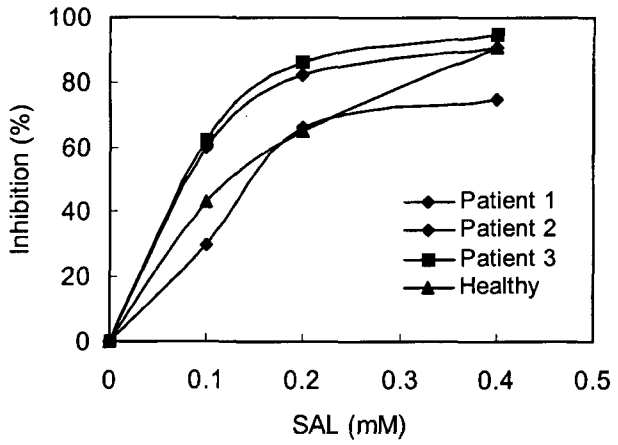

Fig. 4. The inhibitory activity of D-saccharic acid 1,4-lactone on the fecal β-glucuronidase activities of patients with colon cancer. SAL, D-saccharic acid 1,4-lactone.

of colon cancer.

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