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Effect of Chinese Yam on Benzo[a]pyrene Hydroxylase Activities in Rats Fed Dietary Benzo[a]pyrene

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Abstract

To investigate the effect of yam on the activity of benzo[a]pyrene hydroxylase (BPH), the key enzyme associated with polycyclic aromatic hydrocarbons (PAHs) metabolism, rats were fed a fiber free diet for 7 days, whereupon they were switched to experimental diets for another 7 days. Diets contained benzo[a]pyrene (BP, 400 mg/kg diet) and 25% or 50% yam powder (freeze dried and hot air dried). Diets containig pectin and cellulose were compared with diets containing yam. BPH activities were assessed in the liver, lung, kidney, stomach, small intestine and large intestine of rats. BP induced BPH activities in various tissues; 8 fold in liver, 28 in lung and stomach, and 32 in large intestine. The addition of yam significantly lowered BPH activity in liver, lung and stomach, and hot air dried yam was more effective than freeze dried yams. These data suggested that yam containing diet may influence carcinogen metabolism in liver and extrahepatic target tissues by altering activities of BPH and may reduce exposure of these tissues to dietary carcinogens.

Key words: Chinese yam, benzo[a]pyrene hydroxylase

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), of which benzo [a]pyrene is the most commonly studied, are formed by the incomplete combustion of organic matter. They are widely distributed in the environment and human exposures to them is unavoidable (1). PAHs are themselves chemically inert and hydrophobic. However, they undergo metabolic activation in mammalian cells by one of the cytochrome P-450 monooxygenases, benzo[a]pyrene hydroxylase (BPH), to diol-epoxides that bind covalently to DNA causing errors in DNA replication and mutations that initiate the carcinogenic process (2,3). Experimental studies demonstrate that oral administration of benzo[a]pyrene to rodents produces benzo[a]pyrene-DNA adducts in the liver, stomach, colon, and intestine (4) and cancers of the esophagus, forestomach, intestine, lungs, and mammary gland through the induction of BPH (5).

Chinese yam has been used for food and medicine because of its health effects. Although the major component of Chinese yam is starch, the noticeable component is a viscous material. Misaki et al. (6) reported that the mucilage is composed of 71.5% protein and 13.8% carbohydrate, while Tomoda et al. (7) reported 64% protein, 26.2% mannan, 2.7% acetyl group, and 1.2% phosphorous. Because of the adsorption properties of mucilage, the yam had excellent abilities in eliminating heavy metals (8). But the viscosity of the mucilage decreases sharply above 60°C (9). In previous work (10), we found that freeze dried yam was more effective than hot air dried yam in reducing the cholestrol level both in rat plasma and in liver.

The aim of this study is to investigate whether Chinese yam, freeze-dried (FDY) or hot air-dried (HDY), has the ability to reduce carcinogenic activity of benzo[a]pyrene by modulating detoxifying enzymes in tissues, and also to compare the effect of yam with dietary fibers, pectin and cellulose.

MATERIALS AND METHODS

Preparation of Chinese yam powder

Chinese yam was harvested in 1997 at Andong, Kyungbuk. The yams were washed, peeled, and sliced to a 2-mm thickness. One portion of the yam was dried at 70°C in an air-forced drying oven overnight (Hot air-dried yam, HDY), and the other was dried in a freeze dryer (Freeze-dried yam, FDY). Both dried yams were ground with a Cyclotec 1093 Sample Mill (Foss Tecator, Sweden) and screened with a 100-mesh sieve.

Animals and diets

Male Sprague-Dawley rats, aged 6 wk and weighing 150 ±10 g, were used. Rats were randomly assigned to one of 6 experimental diets (8 rats per diet) after 7 days of consuming the AIN-76 based fiber-free diet, then were fed the assigned experimental diets (Table 1) for 7 days. Rats were individually maintained in stainless steel, wire-bottomed cages at 22± 2°C with 12h of light per day and ad libitum access to food and water. The experimental diets were based on the AIN-76 and corrections were made in the quantities of starch and fiber. Chinese yam powder has about 11.0% dietary fiber (4.8% soluble, 6.2% insoluble). All the experimental diet, except fiber free-BP diet, contained 400 mg BP/kg diet.

Sample collection and tissue preparation

The liver, lung, kidney, stomach, small intestine (30 cm of

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Table 1. Composition of experimental diets (g/kg diet)

Ingredients	Fiber free		Freeze-dried yam (FDY)		Hot air-dried yam (HDY)		Pectin	Cellulose
	-BP	+BP	25%+BP	50%+BP	25%+BP	50%+BP	+BP	+BP
Casein	200	200	200	200	200	200	200	200
Corn starch	500	500	250	0	250	0	450	450
Sucrose	200	200	200	200	200	200	200	200
Corn oil	50	50	50	50	50	50	50	50
Vitamin mix[1)]	10	10	10	10	10	10	10	10
Mineral Mix[2)]	35	35	35	35	35	35	35	35
Methionine	3	3	3	3	3	3	3	3
Choline	2	2	2	2	2	2	2	2
Yam powder	-	-	250	500	250	500	-	-
Pectin	-	-	-	-	-	-	50	-
Cellulose	-	-	-	-	-	-	-	50
Benzo[a]pyrene	-	0.4	0.4	0.4	0.4	0.4	0.4	0.4

[1)]AIN-76 vitamin mixture, Teklad, Madison, WI
[2)]AIN-76 mineral mixture, Teklad, Madison, WI

the proximal) and large intestine were collected and homogenized in four volumes of ice-cold 0.25 M sucrose using a Polytron tissue homogenizer (Kinematica, Switzerland). Microsomes were obtained from centrifugation of a postmitochondrial fraction at 100,000×g for 45 min at 4°C, washed and resuspended in phosphate buffer, at pH 7.4, frozen immediately and stored at -80°C.

Biochemical assay

The assay for the reaction catalyzed by benzo[a]pyrene hydroxylase was a modification of the procedure of Nebert and Gelboin (11). The 3-hydroxy-benzo[a]pyrene was determined spectrofluorometrically (Jasco FP-770 spectro-fluorometer) with excitation of 396 nm and emission of 522 nm. Microsomal protein concentrations were determined according to the method of Bradford (12) using a Biorad assay kit.

Statistical analysis

All statistical analysis were performed using the SPSS⁺PC package. Data were expressed as means±SD. Data were subjected to ANOVA, and all groups were compared to one another using the Duncan's multiple range test (α=0.05).

RESULTS AND DISCUSSION

Diet intake and weight change in rats fed various diets for 7 days are shown in Table 2. There were no significant effects of BP intake on diet intake and weight gain compared to fiber free diets with or without added BP. Rats given a diet containing yam or pectin had significantly lower diet intake compared to those given fiber free diet. And the inclusion of freeze-dried yam into the diet resulted in significantly lower weight gain. Ikegami et al. (13) have shown that rats given a diet containing viscous polysaccharides, such as sodium alginate or guar gum, had significantly lower weight gain compared with those given a cellulose diet. It is postulated that the viscosity of the dietary materials (pectin and freeze dried yam) reduced diet intake due to slower motility of the stomach.

The relative weights of the livers (Table 3) were significantly lower in groups fed 50% freeze dried yam (50%FDY), but those of the stomach and cecum were significantly higher. And rats fed pectin were also significantly higher in the weight of the cecum. Rats given a diet containing hot air-dried yam (HDY) showed a trend of low weight of the liver and lung, and high stomach and cecum weights, but were not significant. Fecal weights were significantly increased by the intake of 25%FDY (6 folds), 50%FDY (12 folds), 50%HDY (4.5 folds) or cellulose (4 folds) compared to the intake of fiber free+BP.

The influence of yam and dietary fibers on BPH activities in various tissues of rats were shown in Fig. 1. Liver has the greatest capacity for metabolism of foreign compounds. But tumors induced by various carcinogens, particularly aromatic hydrocarbons, most often involve nonhepatic organs, and the influence of diet component on xenobiotic metabolizing enzyme in these tissues has not been studied extensively. Accordingly, the present study examines the influences of yam

Table 2. Diet intake, weight gain and food efficiency ratio (FER) of rats maintained on experimental diets for 7 days

	Fiber free		Freeze-dried yam (FDY)		Hot air-dried yam (HDY)		Pectin	Cellulose
	-BP	+BP	25%+BP	50%+BP	25%+BP	50%+BP	+BP	+BP
Diet intake (g/day)	17.99±0.91[1)d2)]	17.06±0.65[cd]	14.95±0.63[b]	13.14±2.17[a]	15.71±2.79[bc]	15.10±1.08[b]	14.77±1.15[ab]	15.87±0.73[bc]
Weight gain (g/day)	7.23±0.28[bc]	6.98±0.63[bc]	6.82±0.63[bc]	5.36±1.77[a]	6.13±1.07[ab]	7.55±0.83[c]	7.43±1.27[bc]	6.43±0.93[abc]
FER (%)	40.40±2.07[a]	40.99±4.09[a]	45.51±2.71[ab]	40.17±9.73[a]	40.01±9.43[a]	49.94±2.91[b]	50.66±10.50[b]	40.76±7.48[a]

[1)]Values are means±SD of 8 rats per group.
[2)]Within a row, values not sharing the same superscript letters are significantly different at $p<0.05$ by Duncan's multiple range test.

Table 3. Relative organ weights and wet fecal weights of rats fed experimental diets for 7 days

	Fiber free		Freeze-dried yam (FDY)		Hot air-dried yam (HDY)		Pectin	Cellulose
	-BP	+BP	25%+BP	50%+BP	25%+BP	50%+BP	+BP	+BP
Organs (g/100 g body weight)								
Liver	$3.87\pm0.32^{1)a2)}$	3.94 ± 0.46^{a}	3.52 ± 0.26^{ab}	3.30 ± 0.25^{b}	3.56 ± 0.15^{ab}	3.53 ± 0.43^{ab}	3.86 ± 0.36^{a}	3.95 ± 0.48^{a}
Lung	0.54 ± 0.04^{ab}	0.58 ± 0.03^{a}	0.50 ± 0.05^{a}	0.49 ± 0.07^{a}	0.52 ± 0.05^{ab}	0.50 ± 0.06^{a}	0.56 ± 0.07^{ab}	0.56 ± 0.04^{ab}
Kidney	0.86 ± 0.07^{a}	0.81 ± 0.04^{ab}	0.80 ± 0.03^{b}	0.82 ± 0.05^{ab}	0.83 ± 0.05^{ab}	0.87 ± 0.06^{a}	0.83 ± 0.03^{ab}	0.81 ± 0.05^{ab}
Stomach	0.59 ± 0.04^{a}	0.59 ± 0.06^{a}	0.63 ± 0.06^{ab}	0.72 ± 0.04^{c}	0.68 ± 0.05^{bc}	0.64 ± 0.05^{ab}	0.63 ± 0.05^{ab}	0.61 ± 0.03^{a}
Cecum	0.32 ± 0.10^{a}	0.36 ± 0.12^{ab}	0.40 ± 0.06^{ab}	0.64 ± 0.12^{d}	0.49 ± 0.05^{bc}	0.41 ± 0.02^{ab}	0.55 ± 0.20^{cd}	0.29 ± 0.02^{a}
Feces (wet, g)	0.56 ± 0.13^{a}	0.59 ± 0.21^{a}	3.49 ± 0.95^{b}	7.29 ± 2.05^{c}	1.25 ± 0.33^{a}	2.66 ± 0.72^{b}	0.93 ± 0.29^{a}	2.57 ± 0.30^{b}

[1)]Values are means±SD of 8 rats per group.
[2)]Within a row, values not sharing the same superscript letters are significantly different at $p<0.05$ by Duncan's multiple range test.

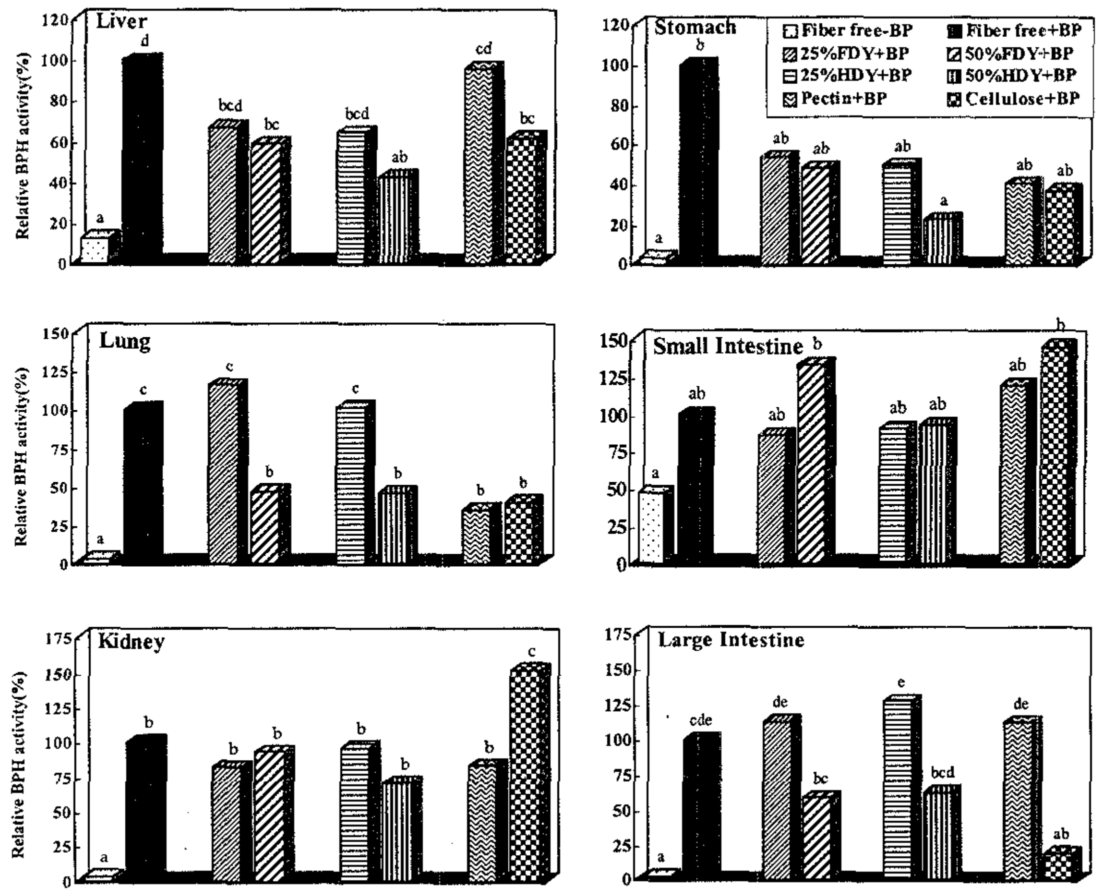

Fig. 1. Relative benzo[a]pyrene hydroxylase (BPH) activities in various tissues of rats fed experimental diets for 7 days.

(freeze dried and hot air dried) on the activities of key enzyme associated with BP bioactivation in the liver and selected extrahepatic tissues known to be targets of BP. Consideration of enzyme activities in target tissues is important because biotransformation in extrahepatic organs is a critical determinant of tissue susceptibility to the toxicants. Rats fed fiber free+BP showed significantly higher BPH activities in all the tissues except the small intestine than those fed fiber free-BP. The induction of BPH activity of rats by BP intake showed about an 8 fold increase in the liver, 28 fold of each in the lung and stomach, 32 fold in the large intestine. The small intestine was also induced by about 2 fold, but this was not significant. Yam or dietary fiber containing diet altered BPH in all the organs except small intestine. Rats fed yam lowered BPH activity about 60% in 50%HDY, 40% in 50%FDY in liver. Cellulose also lowered the enzyme activity about 40% in the liver. BPH activity in the lung was lowered by more than 50% in both yam and dietary fiber containing diets. In contrast to other organs, BPH activity in the kidney was increased by about 1.5 fold in the cellulose diet, and not altered by the other test diets. Activity of BPH in stomach was significantly lowered (about 80%) by the 50%HDY diet, and more than 50% by 50%FDY, pectin and cellulose diet, but this was not significant. BPH activity in the small intestine was not altered by the test diets. In the large intestine, the enzyme activity was significantly lowered (about 80%) only by intake of the cellulose containing diet, and decreased by about 40% with the 50% yam containing diet, but this was not significant.

Considerable effort has been devoted to studying the influence of diet on the incidence of various cancers. Although specific biochemical mechanisms relating diet to carcinogenisis are not clear, it is generally accepted that bioactivation of many carcinogens including polycyclic aromatic hydrocarbons is necessary to elicit their carcinogenic activities (14). A possible link between high consumption of smoked food, which has an abundance of PAHs, and the incidence of stomach cancer was first noted among the population of Iceland (15). And recently, direct evidence for the formation of BP-DNA adducts in human colon *in vivo* has been obtained (16). Kiyohara et al. (17) observed that high BPH inducibility may be strongly associated with the susceptibility to lung carcinogenesis, because BPH is responsible for the activation of BP and other PAHs. Clinton and Visek (18) investigated the ability of dietary fiber to limit exposure of the intestinal epithelium to BP, and observed the inhibition in BP-induced intestinal BPH activity with 10% wheat bran diet. Wheat bran has been one of the most effective fiber sources in decreasing transit time. More rapid movement of intestinal contents through the small intestine may also reduce the time available for PAH absorption, thereby reducing enterocyte exposure and subsequent enzyme induction. Hill (19) suggested that gel-forming fibers, like pectin or guar, which "trap" substrates and increase the fecal loss of steroids, lipids, and other toxicants. Kawata et al. (20) investigated the effect of dietary fiber on the induction of cytochrome P4501A1 in rat colonic mucosa after a single intragastric injection of 3-methylcholanthrene. They found that wheat bran diet inhibited the induction of the enzyme, and suggested that dietary fiber can affect the induction of cytochrome P4501A1 in colonic mucosa by dietary inducers.

Our data indicated that the addition of 50% yam into the diet significantly inhibited BPH induction by BP in the liver, lung and stomach, and hot air dried yam was more effective than freeze dried yams. These data raise the interesting possibility that yam containing diet may influence carcinogen metabolism in the liver and extrahepatic target tissues by altering activities of BPH. But the mechanism underlying these effects are unclear and their significance in protecting against PAH toxicity or carcinogenicity require further study.

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