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Dietary Fiber Reduces Benzo[a]pyrene Hydroxylase Induced by Dietary Benzo[a]pyrene

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Abstract

This study was conducted to determine if dietary fiber would reduce exposure of the tissues to dietary benzo[a]pyrene (BP), a well-known carcinogenic polycyclic aromatic hydrocarbon, as evaluated by benzo-[a]pyrene hydroxylase (BPH) activity. The effects of three different sources of dietary fiber (pectin, poly-dextrose, and cellulose) on BPH activity were studied using Sprague-Dawley rats. In this study, male rats were fed a fiber-free purified diet for 7days, whereupon they were switched to experimental diets for 48h. After 48h, their liver, stomach, small intestinal mucosa and large intestinal mucosa were assayed for BPH activity. Tissues exposed to benzo[a]pyrene (400 mg/kg diet, fiber-free) showed significant increase in the activity of BPH; 27 times in liver, 7 times in stomach, 18 times in small intestinal mucosa and 3 times in large intestine. The inhibition in BP-induced BPH activity by dietary fiber in liver, stomach and small intestinal mucosa was observed in the decreasing order: 10% pectin> 10% polydextrose> 5% polydextrose> 10% cellulose. Decreased BPH induction indicates that soluble dietary fibers, especially pectin and poly-dextrose in this study, protect the tissues of digestive system from exposure to BP.

Key words: pectin, polydextrose, cellulose, benzo[a]pyrene hydroxylase

INTRODUCTION

Epidemiologic and laboratory studies suggest that a number of diseases of the digestive tract including colon cancer are more common in populations consuming diets low in fiber (1-3). The long-term effects of low vs. high fiber intake on the toxic effects of food components, food contaminants, and in general environmental toxic substances can have major implications on the etiology of some diseases and more specifically on the effects of carcinogens (4). It is possible that dietary fiber protects intestinal cells by binding carcinogens to enhance their excretion. And the alteration of the endogenous mixed-function oxygenase (MFO) system, which controls carcinogen activation and detoxification, could be involved in the protective effect of dietary fiber against colon carcinogenesis (5-7). The results of such studies vary with the fiber type and the characteristics and mode of action of the toxin or carcinogen. The polycyclic aromatic hydrocarbons (PAH) are environmental toxins and carcinogens produced during combustion of organic materials (8). Individuals are exposed to high concentrations of PAH from cigarette smoke, charcoal-broiled or smoked meats (9-11). Benzo[a]pyrene (BP) has been the most extensively studied PAH, a powerful carcinogen, and common in the environment.

Because of the possible role of the polycyclic hydrocarbon hydroxylase system in altering the carcinogenic response of tissues to polycyclic hydrocarbon carcinogens, there is considerable interest in the nature of factors which affect the level of activity of this system. The aim of this study is to investigate the ability of different dietary fibers to reduce exposure of the tissues to benzo[a]pyrene. Induction of benzo[a]pyrene hydroxylase (BPH), which is the primary BP-metabolizing enzyme in the MFO system, by dietary BP in rats was used as a biological indicator.

MATERIALS AND METHODS

Experimental diets

The experimental diets were based on the AIN-76 and contained 0, 5, or 10% of dietary fiber with 0 or 400 mg BP/kg diet. Three different kinds of dietary fibers were

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used: cellulose (Sigma Co., St. Louis, MO, USA), pectin (Sigma Co., St. Louis, MO, USA) and polydextrose (Cultor Food Science, Korea). Table 1 shows the experimental diet composition.

Experimental animals and experimental design

Male Sprague-Dawley rats, aged 8 wk and weighing 200±10 g, were used. Rats were randomly assigned to one of 14 experimental diets (five rats per diet) after 7 days of consuming the AIN-76 based fiber-free diet, then were fed the assigned experimental diets for 48h. Feed jars were removed 3h before sacrifice. 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.

Sample collection and tissue preparation

Livers were quickly removed and rinsed several times in ice-cold 0.15 M KCl-0.25 M potassium phosphate buffer, pH 7.25. Nonhepatic tissues were treated similarly, except that the pH of the buffer is 7.55. The small (proximate 30 cm) and the large intestinal mucosa were collected. Tissues were homogenized in ten volumes of the buffer with a Polytron tissue homogenizer (Kinematica, Switzerland) for 30 sec at setting No. 5. Tissue homogenates were centrifuged at 9,000×g for 20 min. The supernatant fluids were collected, 0.2 vol of 0.1M $CaCl_2$ in 0.25 M sucrose were added to each, and the samples were kept on ice for 30 min. Microsomal pellet were obtained after further centrifugation at 20,000×g for 30 min. Microsomal pellets were washed and resuspended with buffer and stored at -70°C for enzyme assay.

Table 1. Composition of experimental diets

Ingredient	Fiber-free	Pectin		Cellulose		Polydextrose	
		5%	10%	5%	10%	5%	10%
Casein	200	200	200	200	200	200	200
Corn starch	200	150	100	150	100	150	100
Sucrose	500	500	500	500	500	500	500
Corn oil	50	50	50	50	50	50	50
Vitamin mix[1]	10	10	10	10	10	10	10
Mineral mix[2]	35	35	35	35	35	35	35
Methionine	3	3	3	3	3	3	3
Choline	2	2	2	2	2	2	2
Pectin	-	50	100	-	-	-	-
Cellulose	-	-	-	50	100	-	-
Polydextrose	-	-	-	-	-	50	100
Benzo[a]pyrene	0 or 0.4	0 or 0.4	0 or 0.4	0 or 0.4	0 or 0.4	0 or 0.4	0 or 0.4

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

Biochemical assay

The assay for the reaction catalyzed by benzo[a]-pyrene hydroxylase was a modification of the procedure of Nebert and Gelboin (12). The 3-hydroxybenzo[a]-pyrene was determined spectrofluorometrically (Jasco FP-770 spectrofluorometer) with excitation of 396nm and emission of 522nm. Microsomal protein concentrations were determined according to the method of Bradford (13) using Biorad assay kit.

Statistical analysis

All statistical analyses were performed using SPSS $^+$PC package. Data were expressed as means±SEM. Data were subjected to ANOVA, and all groups were compared with one another using the Duncan's multiple range test.

RESULTS

Diet intake and weight change in rats fed various dietary fiber with or without BP for 48h were shown in Table 2. The amount of BP intake was obtained by multiplying diet intake by 0.4. Cellulose group showed increased diet intake compared to the other groups, and that 400 mg BP/kg diet did not influence diet intake. It is postulated that the lower energy-density of cellulose diet increased diet intake, and the viscous dietary fiber slightly reduced diet intake due to the slower motility of the stomach. The amount of BP intake during the experimental periods was highest in rats fed cellulose.

Weight change was not significantly different among the groups except cellulose without BP group which is significantly higher in weight gain than others. Weight gain was slightly inhibited by BP in the diet, and rats given a diet containing pectin or polydextrose had significantly lower in weight gain than those given a cellulose diet.

The activity of BPH in the tissues of rats was shown in Table 3. Rats fed BP showed significantly higher BPH activity than those not fed BP. The induction of BPH activity of rats fed BP compared to non-BP group was

Table 2. Feed intake, benzo[a]pyrene (BP) intake and body weight change

Groups	Feed intake(g/48h)	BP intake(mg/48h)	Weight change(g/48h)
Fiber-free			
-BP	30.88±3.54^{ab}	0	5.78±4.48^{ab}
+BP	30.62±2.59^{ab}	12.25±1.04^{bc}	1.00±4.08^{ab}
5% fiber			
Pectin			
-BP	22.65±3.14^{b}	0	7.40±6.54^{ab}
+BP	24.47±1.67^{b}	9.79±0.67^{ab}	2.40±4.37^{ab}
Cellulose			
-BP	49.83±1.51^{c}	0	15.20±0.49^{c}
+BP	39.89±6.33^{ac}	15.95±2.53^{c}	10.00±6.99^{bc}
Polydextrose			
-BP	26.14±3.67^{ab}	0	5.33±1.82^{ab}
+BP	24.79±2.66^{ab}	9.92±1.06^{ab}	2.83±3.23^{ab}
10% fiber			
Pectin			
-BP	27.18±3.43^{ab}	0	6.80±6.10^{ab}
+BP	23.57±3.21^{ab}	9.43±1.28^{ab}	-2.00±6.89^{ab}
Cellulose			
-BP	46.82±1.52^{c}	0	14.40±1.29^{c}
+BP	37.14±5.80^{ac}	14.86±2.32^{c}	3.33±4.92^{ab}
Polydextrose			
-BP	25.51±2.98^{ab}	0	2.33±4.63^{ab}
+BP	22.27±2.87^{b}	8.91±1.15^{ab}	-4.20±18.96^{ab}

Values are mean±SEM

Different superscript within the same column is significantly different ($p<0.05$) by Duncan's multiple range test.

Table 3. Relative benzo[a]pyrene hydroxylase (BPH) activity in rats fed dietary fiber with or without benzo[a]pyrene for 48h

Groups	Relative BPH activity(%)			
	Liver	Stomach	Small intestine	Large intestine
Fiber-free				
-BP	100±26^{a}	100±11^{a}	100±28^{a}	100±9^{a}
+BP	2760±446^{c}	712±197^{b}	1862±165^{b}	288±92^{b}
5% fiber				
Pectin				
-BP	78±38^{a}	184±29^{a}	68±21^{a}	98±14^{a}
+BP	2287±843^{c}	1148±340^{b}	2027±461^{b}	278±59^{b}
Cellulose				
-BP	63±236^{a}	127±27^{a}	61±18^{a}	131±15^{ab}
+BP	1692±233^{c}	1333±322^{b}	2761±661^{b}	249±79^{ab}
Polydextrose				
-BP	90±24^{a}	135±29^{a}	67±18^{a}	69±8^{a}
+BP	492±62^{b}	482±47^{b}	1730±215^{b}	562±138^{c}
10% fiber				
Pectin				
-BP	43±19^{a}	142±33^{a}	85±22^{a}	112±10^{a}
+BP	66±35^{a}	481±139^{a}	186±95^{a}	243±52^{ab}
Cellulose				
-BP	117±55^{a}	74±12^{a}	64±12^{a}	120±21^{a}
+BP	1142±382^{bc}	927±345^{b}	2344±471^{b}	223±47^{ab}
Polydextrose				
-BP	86±31^{a}	114±22^{a}	67±20^{a}	55±6^{a}
+BP	439±139^{ab}	609±174^{b}	1371±385^{b}	404±129^{c}

Values are mean±SEM

Different superscript within the same column is significantly different ($p<0.05$) by Duncan's multiple range test.

27 folds increase in liver, 7, 18 and 3 folds in stomach, small intestine, and large intestine, respectively. Various types of dietary fibers without BP did not influence BPH activity. Rats fed 5% pectin or 5% cellulose with BP did not show any difference in lowering BPH induction after exposure to BP. But the addition of 5% polydextrose showed significant inhibition in hepatic BPH induced by BP intake. With BP in the diet, 10% pectin significantly reduced BPH activity in most tissues, but 10% polydextrose reduced it only in hepatic tissue. Pectin (10%) was effective fiber source in reducing the induction of BPH activity in most tissues. In large intestinal mucosa, cellulose might be effective in reducing BPH induction, but polydextrose significantly induced BPH ($p<0.05$).

DISCUSSION

The environmental factors markedly influences the incidence of cancer. In most cases specific causal agents are unknown, but the agents may be related to life-style, diet or exposure to naturally occurring or man-made environmental carcinogens, such as pesticides, food contaminants, or pollutants. Studies from numerous laboratories indicated that the mixed-function oxidase systems (MFOs) represent the key enzymologic interface between humans and foreign chemicals (14-16), and an initial metabolic barrier to noxious foreign chemicals in the external environment. PAHs are ubiquitous pollutants of air, soil and water, and are major carcinogenic components of cigarette smoke. A correlation has been established between the DNA binding of PAHs and their carcinogenicity (17). One of the most abundant PAHs is BP, and a key reaction in both detoxification and activation of BP is catalyzed by BPH, which is an MFO (18). Thus BPH may be used for determining susceptibility to PAH carcinogenesis specifically and possibly to other organic chemical carcinogens as well. Because the activity of BPH is inducible in 16h after exposure to various drugs and chemicals and is found in most animal tissues (12,19,20), we have given BP for 48h and observed

the ability of various types of dietary fibers to reduce exposure of tissues to BP.

The incorporation of BP into diets resulted in large increase in BPH activity after 48h exposure. The addition of 10% pectin significantly inhibited BPH induction by BP in most tissues except large intestine. Polydextrose is less effective in inhibiting the BPH induction than pectin but more effective than cellulose. These results support the hypothesis that soluble fiber reduces exposure of tissues to the carcinogen (21). Several mechanisms whereby dietary fiber decreases tissue exposure to BP can be postulated based upon presumed effects of digestive processes on absorption of lipophilic compounds (22). Dietary fiber may inhibit pancreatic digestive enzyme activity, including lipolysis, thereby slowing formation of mixed micelles and retarding or delaying the absorption of BP and other PAH (23). Fiber may also inhibit the solubilization of dietary BP into micelles by direct binding. Wheat bran binds 7,12-dimethylbenz(a) anthracene in simple bile salt solutions *in vitro* (24), and various forms of fiber have also been reported to bind other, more water soluble carcinogens, such as 1,2-dimethylhydrazine (25) and N-nitrosodiethylamine (26). Story and Kritchevsky. (27) also showed that fiber binds bile acids but this varies with fiber sources. *In vivo* absorption of strongly nonpolar compounds, similar to PAH, was minimal in the absence of bile acids. Other studies have shown that increased excretion of bile acids in the presence of dietary fiber reduces the enterohepatic bile acid pool, causing a reduction in the bioavailability of dietary lipids, and may affect the absorption of dietary PAHs (28,29). Ikegami et al. (30) reported that pentachlorobenzene residues in the liver, kidney and adipose tissues were much lower in rats fed viscous indigestible polysaccharides than in rats fed cellulose. They supported that viscous indigestible polysaccharides accelerate the metabolism and excretion of lipophilic xenobiotics and decreases their accumulation in the body. Hill (31) found that gel-forming fibers, like pectin or guar, which "trap" substrates and increase the fecal loss of steroids, lipids but both cellulose and lignin had no effect.

In summary, we observed that 10% pectin reduced the induction of BPH activity in most tissues except in large intestine, and 10% polydextrose in hepatic tissue, suggesting a protective effect of soluble dietary fibers against dietary BP exposure. But its protective action against carcinogen was different according to the types and the doses of dietary fiber. It is speculative that the long-term effects of exposure to PAHs in rats or humans have the same results. The mechanisms underlying these effects are unclear and their significance in protecting against PAH toxicity or carcinogenicity require further study.

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