Effect of Soy Yogurt (Lactic Acid-Fermented Okara in Soymilk) on Intestinal Polyamine Level in Rats

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Polyamines, putrescine, spermidine and spermine, are important constituents of all mammalian cells and are essentially involved in a variety of regulatory step during normal and malignant cell proliferation. Okara and soymilk fermented with *Lactobacillus* were combined to form soy yogurt, which is rich in polyamine content. We investigated the effect of soy yogurt on polyamine levels in intestines and blood of rats. The ingestion of soy yogurt induced the increase of mucosal polyamine level in small intestine. Although the polyamine level in cecum was increased by the ingestion of soy yogurt, the polyamine levels in large intestine and blood were not increased. It was found that the ingestion of polyamine-rich soy yogurt could affect the polyamine level in only small intestinal mucosa. The large intestine seems to regulate the polyamine level to prevent own cells from hyperproliferation. It was assumed that blood kept polyamine level constant in spite of the level changes in intestines.

Keywords: polyamine, okara, soymilk, lactic fermentation, soy yogurt, intestinal mucosa, cecum

Introduction

Okara is a byproduct generated mainly during processing of soybean. Although a great deal of okara has been treated as an industrial waste, it contains functional components including soy protein (Anderson *et al.*, 1995, Fukui *et al.*, 2002, Higaki *et al.*, 2006, Iwami *et al.*, 1986, Makino *et al.*, 1988, Nagata *et al.*, 1982, Takagi *et al.*, 2008, Tanaka *et al.*, 1989), isoflavone (Sung *et al.*, 2004), dietary fiber (O'Toole, 1999) and polyamine (Bardocz *et al.*, 1993). We examined the beneficial use of okara supplemented in lactic acid-fermented soymilk (soy yogurt). We already investigated the effects of soy yogurt on plasma and hepatic lipid profiles in rats (Kitawaki *et al.*, 2007) and found that soy yogurt exhibits lipid-metabolism modification (Kitawaki *et al.*, 2009).

In a previous paper, we reported the increase of polyamine content in okara and soymilk by lactic fermentation (Takagi *et al.*, 2009). The polyamines, putrescine, spermidine and spermine, are important constituents of all mammalian cells and are essentially involved in a variety of regulatory steps

during normal, adaptive, and malignant cell proliferation (Larqué *et al.*, 2007, Pegg, 1988, Pegg and McCann, 2002, Tabor and Tabor, 1984). The polyamine level is correlated with biomarkers of proliferation in the large intestinal mucosa where dysregulated epithelial hyperproliferation is associated with large intestinal cancer risk (Wolter *et al.*, 2004). Regulation of polyamine biosynthesis prevents overproduction and deficiency of polyamine in animals, and excessive amounts of polyamine are harmful, potentially leading to cancer (Pegg, 1988). The influence of dietary polyamines on human and animal are completely unknown. Wang *et al.* (2000) reported that dietary soy protein is associated with reduced intestinal mucosal polyamine level in rats. However, we observed the distinct response of the polyamine level between the small and large intestines by the ingestion of soy yogurt. In the present study, we investigated the effect of soy yogurt on polyamine levels in intestinal mucosa and blood.

Materials and Methods

Animals Male Sprague-Dawley (SD) rats (n=12) aged 7 weeks, body weight 200–220 g (CLEA Japan, Inc. Japan), were housed individually in raised stainless steel cages with

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Table 1. Composition of experimental diet (weight %).

Ingredients	Diet Group	
	C (AIN-93G)	S
Corn starch	39.7	37.2
Casein	20.0	11.4
α -Corn starch	13.2	13.0
Granulated sugar	10.0	10.0
Soy oil	7.0	2.5
Cellulose powder	5.0	0.8
Mineral mix (AIN-93G-MX)	3.5	3.5
Vitamin mix (AIN-93-VX)	1.0	1.0
<i>L</i> -Cystine	0.3	0.3
Choline bitartrate	0.25	0.25
<i>tert</i> -Butylhydroquinone	0.0014	0.0014
Dried soy yogurt	0.0	20
Total	100	100

C: control, S: soy yogurt

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Table 2. Composition of freeze-dried soy yogurt.

Component	%
Water	5.0
Protein	37.3
Fat	23.6
Carbohydrate	8.1
Dietary fiber	21.1
Mineral	4.9

wire-mesh floors and fronts under controlled conditions of light (12-h light/dark cycle), humidity (55±7%) and temperature (23±1°C). Food and water were consumed *ad libitum*.

Diets The diet was based on the AIN-93G diet formula. Rats were fed AIN-93G on a pre-feeding term. The rats were divided into two groups, control group and soy yogurt group. Control group was fed AIN-93G diet. Soy yogurt group was fed AIN-93G replaced with 20% soy yogurt, as shown in Table 1. To make soy yogurt, soymilk and powdered okara were blended in a ratio of 2 to 1 on a dry basis and fermented using *Lactobacillus delburickii* subsp. *Delburickii* strain of SNC33 at 40°C for 15 h. The nutritional composition of dry soy yogurt is shown in Table 2. Diet of each group had equal nutritional composition and was administered for 4 weeks.

Intestines After the feeding period, non-fasted rats were sacrificed without affliction by diethyl ether anesthesia to collect the intestine. The entire intestine was excised immediately and placed into ice-cold 10 mmol/l PBS, pH 7.4. Intestinal mucosa was gently scraped with a microscope slide from each segment and stored at -80°C according to the method of Wang *et al.* (2000) until polyamine analysis. This animal experiment was performed according to the guideline of the Animal Use Committee of Mukogawa Women's University.

Determination of polyamine Mucosal sample was suspended in PBS and homogenized. After removal of gross

debris by centrifugation, 1,12-dodecanediamine was added as an internal standard, and then proteins were precipitated with a final 0.5 mol/L perchloric acid. The protein-free supernatant (500 μ l) was mixed with 350 μ l of saturated sodium carbonate and 400 μ l of 37 mmol/L dansyl chloride, then the mixture were incubated at 60°C for 1 h. Dansylated polyamines were extracted in toluene, dried, then redissolved in 100 μ l acetonitrile and finally quantified using HPLC (Wang and Higuchi, 2000). Polyamines from each sample were separated by reverse-phase HPLC with a TSK ODS-80Ts column (4.6×250 mm, Tosoh Corp., Japan) with 10 mmol/L heptanesulfonate buffer, pH 3.4, using an acetonitrile gradient at a flow rate of 0.7 mL/min. Dansylated polyamines were detected using a fluorescence detector with excitation at 330 nm and emission at 470 nm, and the peak areas assigned to an internal standard were used to calculate the polyamine content.

Statistical analysis Analysis involved SPSS, version 12.0j for Windows. Each value is shown as the mean±SE. All data were evaluated by one-way ANOVA and then by Student's t-test at $p<0.05$.

Results and Discussion

Food intake The food intake, initial and final body weights, and food efficiency of rats did not significantly differ between the two diet groups (Table 3). However, the difference of cecum weight depending on the content was significant between the two groups. The cecum weight/body weight of soy yogurt group was higher than that of the control group (Fig. 1).

Polyamine In our previous paper, we reported that the polyamine (putrescine, spermidine and spermine) content of fermented okara/soymilk mixture, i.e., soy yogurt (4.07 nmol polyamines/mg food), was higher compared with that of the unfermented mixture (2.68 nmol polyamines/mg food) (Takagi *et al.*, 2009); spermidine level was especially higher than those of other polyamines. Polyamines are known to play some essential roles in cell proliferation, regeneration and differentiation (Tabor and Tabor, 1984). Matsumoto *et al.* (2006) reported that the intestinal polyamine concentrations of elder volunteers were increased by ingestion of yogurt. In the present study, the level of polyamines (putrescine, spermidine and spermine) in small intestinal mucosa in the soy yogurt group

Table 3. Effect of test food on growth and feed efficiency ratio in rats.

	Control	Soy yogurt
Initial body weight (g)	297.8±4.4	301.1±2.4
Final body weight (g)	453.9±5.5	450.0±9.1
Total food intake (g/4 weeks)	671.7±20.0	689.9±29.8
Food efficiency	0.23±0.005	0.22±0.003

Each value is expressed as the mean ± SE ($n=6$).

Fig. 1. Cecum weight/body weight percent of rats.

Each value is expressed as the mean $\pm$ SE ($n=6$).

C: Control, S: Soy yogurt, *** $p<0.005$

was higher than that of the control group, and polyamines ingested from diets seem to be incorporated immediately after ingestion of polyamine-rich soy yogurt (Table 4). The spermidine and spermine contents in small intestine mucosa showed a significant increase in the soy yogurt group. In the small intestinal mucosa, the spermidine levels were always higher than those of putrescine and spermine. The polyamine level in large intestinal mucosa did not significantly increase (Table 4). The putrescine content in the large intestine of the soy yogurt group showed a significantly low level. Although the total polyamine level in small intestinal mucosa showed a distinct increase in the soy yogurt group, that in large intestinal mucosa of the soy yogurt group did not show any increasing tendency. On the other hand, Wang *et al.* (2000) reported that a soy protein diet lowered polyamine levels in small and large intestinal mucosa. This report supported our results in large intestinal mucosal polyamines, whereas the polyamine level in small intestinal mucosa was different from our results. Increasing the level of polyamines in the diet by soy yogurt intake would lead to incorporation of polyamines in small intestinal mucosa, despite inhibition of polyamine absorption with soy protein. Excess polyamine in large intestinal cells accelerates tumorigenesis (Wolter *et al.*, 2004). The large intestine seems to regulate the polyamine level to prevent its own cells from hyperproliferation.

The cecum polyamine content in the soy yogurt group was higher than that in the control group. Cecum microorganisms in rats that ingested soy yogurt might be increased and polyamine was produced (Table 4), as cecum weight/body weight of the soy yogurt group was significantly higher than that of control group (Fig. 1). As reported in our previous paper (Takagai *et al.*, 2009), the ingestion of guar gum or soy yogurt increased cecum weight and the level of polyamines. *Bifidobacteria* in cecum was increased to more than 100 fold by soy yogurt ingestion, whereas the remarkable increase of *Bacteroides* involved in polyamine synthesis was not detected (unpublished data). However, Noack *et al.* (1998) observed

a vigorous increase of both *Bifidobacteria* and *Bacteroides* in rats that had ingested guar gum. As *Bifidobacteria* scarcely participates in polyamine biosynthesis in rats (Noack *et al.*, 1998), we have not yet specified the bacteria producing polyamines in the cecum. However, in the present study, changes in cecum polyamine content in rats ingested soy yogurt could be affected by various microorganisms.

In the present study, there was no significant difference between the blood polyamine levels of the two groups (Table 4). Brodal *et al.* (1999) also reported that polyamine intake did not exhibit any effect on blood polyamine levels. Moreover, their results suggested that blood polyamine levels were regulated by intestinal absorption and catabolism. Delzenne *et al.* (2000) reported that the high level of polyamine in rat cecum did not affect the level in the portal blood.

These results exhibited that the ingestion of soy yogurt induced the increase of the mucosal polyamine level in small intestine whereas the levels in large intestinal mucosa and blood did not increase. The ingestion of soy yogurt induced the absorption of polyamines in small intestinal mucosa. Thus the increase in mucosal polyamine level should enhance intestinal mucosal barrier function as reported by Guo *et al.* (2003). On the other hand, the large intestine seems to regulate the polyamine level to prevent its own cells from hyperproliferation and tumorigenesis. It was assumed that the blood kept the polyamine level constant despite the level changes in intestinal mucosa. Thus, a large amount of polyamine intake may be required to increase the polyamine level in blood.

Table 4. Polyamine levels in intestine, blood and cecum of rats.

Polyamines	Control	Soy yogurt
Small intestine (nmol/mg protein)		
Putrescine	0.8 $\pm$ 0.2	1.3 $\pm$ 0.2
Spermidine	9.9 $\pm$ 2.0	15.2 $\pm$ 1.3*
Spermine	4.7 $\pm$ 0.6	6.5 $\pm$ 0.3*
Total	15.4 $\pm$ 3.2	23.1 $\pm$ 1.8*
Large intestine (nmol/mg protein)		
Putrescine	1.1 $\pm$ 0.2	0.6 $\pm$ 0.0*
Spermidine	8.6 $\pm$ 0.6	9.2 $\pm$ 0.5
Spermine	5.7 $\pm$ 0.6	5.4 $\pm$ 0.3
Total	15.4 $\pm$ 1.1	15.1 $\pm$ 0.7
Blood (nmol/l)		
Putrescine	2.3 $\pm$ 0.1	2.4 $\pm$ 0.1
Spermidine	2.8 $\pm$ 0.3	2.6 $\pm$ 0.1
Spermine	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
Total	5.3 $\pm$ 0.3	5.3 $\pm$ 0.2
Cecum (nmol/mg cecum content)		
Putrescine	0.4 $\pm$ 0.1	0.1 $\pm$ 0.0*
Spermidine	2.1 $\pm$ 0.2	3.7 $\pm$ 0.2***
Spermine	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0
Total	2.7 $\pm$ 0.3	4.1 $\pm$ 0.2*

Each value is expressed as the mean $\pm$ SE ($n=6$). Asterisks show significantly different values from that of the control by Student's *t*-test. (* $p<0.05$, *** $p<0.005$)

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