ORAL ADMINISTRATION OF SWINE GASTRIC MUCIN ALTERED SERUM NITRATE, CHOLESTEROL AND TRIGLYCERIDE IN MICE

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Background

Mucin is a glycoprotein existed on epithelial cells of digestive and respiratory tracts with various biological activities such as bactericidal, chemotactic and inhibition of cell adhesion. Mucin biosynthesis is known to be augmented by nitric oxide. On the other hand, we had observed that swine gastric mucin activated macrophages and the activated macrophages produced nitric oxide *in vitro* (Miwa et al. 1997).

Objective

The objective of this study was examination of *in vivo* biological activity of dietary mucin. We examined the serum and urine level of nitrite and nitrate, the stable end products of nitric oxide, and the serum concentration of cholesterol and triglyceride of the mice.

Methods

Two groups of male C3H mice, 5week-old mice (young mice) and 14 month-old (old mice) were used. They were fed basal diet for 1 week and then experimental diets with various concentration of gastric mucin from swine for 5weeks. Nitric oxide concentration in serum, urine and liver homogenate was analyzed with NO analyzer as nitrite plus nitrate. Total cholesterol, HDL-cholesterol and triglyceride in serum were measured by the following kits; Cholesterol C-Test Wako, HDL-Cholesterol Test Wako, and Triglyceride G-Wako, respectively. Results were expressed as means ± SE, and analyzed by Student's t-test.

Results and Discussions

The effects of 5-weeks feeding of the swine gastric mucin containing diet on serum nitrate and nitrite level in 5-weeks-old and 72-weeks-old mice are shown in Table 1. Despite of the nitric oxide induction activity of mucin *in vitro*, reduction of serum and liver nitrate and nitrite level was observed with the mucin in a dose dependent manner. The reduction in serum was significant ($P < 0.05$) with the diet containing more than 2% mucin in the young mice and with 5% mucin in the old mice. The nitrate and nitrite level in urine was significantly increased in the control mice during the five-weeks experimental period with unknown reason. The mucin diet seemed to suppress the increase (Fig1). We also examined the serum concentration of cholesterol and triglyceride of the mice, as there were numbers of reports in which nitric oxide production is suggested to correlate with lipid metabolism. The mucin diet also caused a reduction of total triglyceride and cholesterol level and an increase of HDL-cholesterol level in serum (Table 1). Liver and final body weights were unaffected by the dietary mucin.

Table 1. Effect of Dietary Mucin on Serum Nitric Oxide Production and Lipids in Mice

	Young mice[a]			Old mice[b]		
	Basal diet	2% Mucin	5%Mucin	Basal diet	2% Mucin	5%Mucin
Final body weight (g)	29±0.5	29±0.3	28±0.6	38±2	37±1	37±0.2
Liver weight (g)	1.0±0.02	1.0±0.02	0.9±0.02	1.13±0.04	1.11±0.05	1.09±0.02
Nitric oxide						
Serum (μM)	40±3	20±1*	15±1.2*	46±3	45±2	32±2*
Liver (μmol/g tissue)	146±6	108±3*	98±3*	110±6	108±5	105±7
Triglycerides (mmol/l)	1.64±0.03	1.36±0.02*		1.36±0.02	1.34±0.04	1.30±0.03
Total cholesterol (mmol/l)	4.69±0.01	4.01±0.08*		4.36±0.24	4.20±0.05	4.12±0.13
HDL-cholesterol (mmol/l)	2.99±0.03	3.66±0.10*		3.07±0.23	3.17±0.07	3.26±0.03

Values are means ± SE for each treatment . a) n = 6, b) n = 7.
*Significantly different from basal diet by studen's t-test (P<0.05).

Nitric oxide is known to be a multi-functional signal and effecter molecule involving various biological activities, such as immune, vascular, gastrointestinal absorption, neural systems and so on. Among various sources of nitric oxide in whole body, much greater amount of NO is produced by iNOS in defensive mechanism especially at the site of infection and inflammation. Green et al. (1981) reported the elevated nitric oxide production upon infectious diarrhea in human. It suggested that a significant portion of endogenous nitric oxide was synthesized from defense system. On the other hand mucin has also a very important role in defense mechanism especially in gastrointestinal and respiratory tract as a barrier molecule. Nitric oxide, probably as a signal molecule of pathogen invasion, is known to enhance mucin biosynthesis and secretion. This induction mechanism itself suggests the important protective role of mucin. Although we previously observed that swine gastric mucin preparation stimulated the NO induction in macrophage in vitro, the effect of the dietary mucin intake was the reduction of serum nitrite level. As mucin has bactericidal and adhesion inhibition or virus replication inhibition activities, we speculate the in vivo effect accounts for these characters of mucin in gastrointestinal tract. Dietary mucin might suppress the interactions between gastrointestinal epithelial cells and enteric microbes, resulting the low endogenous NO production. We have not determined the reason but the urine level of the NO end products was increased during the experimental period. We speculate the involvement of enteric microbes on it to some extent. The detailed mechanism of this phenomenon must be elucidated further.

We also observed serum cholesterol and triglyceride lowering effect of mucin intake. Numbers of reports have appeared on the topics of the relationship between nitric oxide and cholesterol and/or triglyceride. Our previous study has provided evidence that hypertriglyceridemia and hypercholesterolemia by L-N$^{\omega}$ nitroarginine were mediated through lower fatty acid oxidation and impaired bile acid synthesis respectively (Khedara et al.1996, 1998, 1999). Crespo et al. (1999) reported LPS induction of iNOS and increased serum levels of cholesterol and triglyceride, and iNOS inhibition by melatonin counteracted it.

The suppression of the interactions between gastrointestinal epithelial cells and enteric microbes by dietary mucin might result in the decreased infection signal and consequent reduction of cholesterol and triglyceride. Mucin is also known to directly interact with biliary lipids and accelerate cholesterol gallstones formation. This character of mucin might account for inhibition of lipid and cholesterol to some extent.

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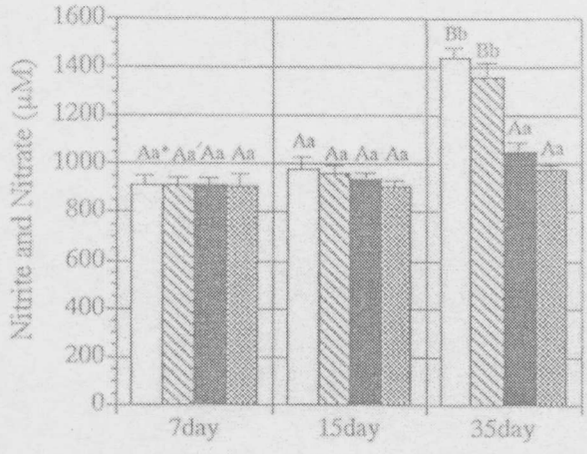

Fig.1 Effect of Different Concentrations of Mucin on Nitric Oxides in Urine

□:Control, ▨: 2% mucin, ■ :3% mucin, ▩: 5% mucin

*Large letter expressed comparison between the concentrations of mucin.
Small letter expressed comparison between feeding days.
Values not sharing a common letter differ significantly at $p < 0.05$ ($n = 6 - 7$).