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Physical changes of chitin and chitosan in canine gastrointestinal tract

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Abstract

Physical changes of chitin and chitosan were evaluated in canine gastrointestinal tract. Chitin and chitosan were packed into nylon bags (3×1 cm^2, 45 μm mesh) and administered orally, and then the bags were recovered from the feces. Chitosan bags were also inserted surgically into the jejunum and colon. The changes in the weight of the bags were evaluated. Chitosan was decreased to $<10\%$ of its original weight and had formed a film, while chitin did not undergo any change in shape or weight. The loss of chitosan was about 40% and the weight loss of chitosan was observed in the colon routes. The weight loss of chitosan in the colon was observed in the presence of colonic contents. An in vitro study was also performed to investigate the physical change of chitosan after treatment with artificial gastric and intestine juices. The in vitro study showed that chitosan became a gel in artificial gastric juice and its weight decreased by 15%, but there were no such physical changes when it was placed in artificial intestinal juice. The present study showed that chitin did not undergo any changes in weight and shape in the gastrointestinal tract, whereas chitosan did. In addition, chitosan was found to be affected in the stomach and large intestine, but not in the small intestine.

Keywords: Chitin; Chitosan; Oral administration; Dogs

1. Introduction

Recently, chitin and chitosan have attracted much interest as functional foods. The cholesterol-lowering action of oral chitosan was first reported by Sugano, Fujikawa, Hiratsuji and Hasegawa (1978) and since then it has been confirmed by several investigators (Fukuda, Fukuda & Ayaki, 1991; Hirano & Akiyama, 1995; Maezaki et al., 1993; Nauss, Thompson & Nagyvary, 1983; Sugano, Fujikawa, Hiratsuji, Nakashima, Fukuda & Hasegawa, 1980; Sugano, Watanabe, Kishi, Izumi & Ohtakara, 1988; Vahouny, Satchithanadam, Cassidy, Lishtfort & Furdy, 1983). On the other hand, chitin does not display a cholesterol-lowering action, although chitin induces higher excretion of triglycerides in feces (Zacour, Siva, Cecon, Bambirra & Vieira, 1992). In spite of much attention to these materials as functional foods, information regarding the digestion and absorption of chitin and chitosan in the gastrointestinal tract is limited. Hirano et al. (1990) indicated that chitin and chitosan were digested 35–83% by rabbits and 88–98% by hens and broilers. Yoshino et al. (1991) found that chitin was degraded in the rumen of sheep, while chitosan was not. It is well known that chitosan dissolves in the acidic digestive fluid of the stomach and coagulates in the alkaline fluid of the intestine. Nishimura, Watanabe, Hong, Takeda, Wada & Yukawa (1997) demonstrated the absorption of chitosan in the gastrointestinal tract using ^{14}C-labeled chitosan. However, it is unclear in which site of the gastrointestinal tract chitin and chitosan are digested. To evaluate chitin and chitosan as functional foods, it is important to investigate what kinds of changes occur in the gastrointestinal tract.

In this study, we evaluated the physical changes of chitin and chitosan in the gastrointestinal tract of dogs.

2. Experimental

2.1. Animals

Sixty-six mongrel dogs 3–5 years old and weighing 9–12 kg were used in this study. All the dogs were healthy according to clinical and hematological tests.

2.2. Reagents

Chitin and chitosan: Chitin and chitosan were supplied by Sunfive Inc. (Tottori, Japan). Chitin was prepared from squid pen as particles with an average diameter of 0.5 or 3 mm. The molecular weight was approximately 3×10^5.

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Chitosan was prepared from the purified chitin of crab shell by chemical deacetylation. Approximately 80% of the deacetylated particles had an average diameter of 0.5 or 3 mm and the molecular weight was approximately 4×10^5. The molecular weight and the degree of deacetylation were determined by the viscosity (Tokura & Nishi, 1995) and IR methods (Shigemasa, Matsuura, Sashiwa & Saimoto, 1996), respectively. Depending on the particle sizes, chitin/chitosan were named as powder and flake types. Cellulose (average diameter: 0.12 mm) was purchased from Wako Pure Chemical (Osaka, Japan).

Artificial gastric and intestinal juices: Artificial gastric juice was made from 2.0 g/l of NaCl (Wako, Osaka, Japan), 3.2 g/l of pepsin (digestive power 1:100, Wako Pure Chemical, Osaka, Japan), and 24 ml/l of 1 N HCl (Wako Pure Chemical, Osaka, Japan). Artificial intestinal juice consisted of 15 g/l of $NaHCO_3$ (Wako Pure Chemical, Osaka, Japan) and 2.8 g/l of pancreatin (starch digestive power >3000–5000 units/g, protein digestive power >26,000–46,000 units/g, fat digestive power >750–1400 units/g, Wako Pure Chemical, Osaka, Japan).

3. Methods

3.1. Nylon bag administration in dogs

In the preliminary experiment, we confirmed a suitable condition for the nylon bag administration using three dogs. We investigated the optimal size of the nylon bag that passes through the digestive tract and also the drying time of the recovered bag. Various sizes (1×0.5–3×3 cm^2) of the nylon bag including the metal ring were made of 300 mesh (pore size 45 μm) nylon fabric (Nytal, No. 282, Swiss). Each dog received three nylon bags orally. The radiographic examination of the abdomen was performed at 6, 12, and 24 h after the oral administration to observe for the passage of the nylon bag through the pylorus. For the determination of the drying time, the bags containing each type of chitin or chitosan (about 150 mg/bag) were immersed in water at 38°C for 24 h and then placed in a drying oven at 38°C. The weight of the bag was measured every 2 h. From the results it was found that the nylon bag with a size of 3×1 cm^2 was most suitable because of its passing through the pylorus within 12 h, which is also the normal time for passage of food through the pylorus. In bags of sizes larger than of 3×1 cm^2, it took 12–24 h to pass through the pylorus. Almost all the bags dried at 4 h and reached the original weight at 6 h. So the drying time was tentatively set as 7 h. When the weight of each bag was compared at 2 h after drying, chitosan was found to retain more water than chitin, and also the powder type retained more water than the flake type in both the chitin and chitosan groups.

3.2. Experiment 1

Sixty dogs were divided into three groups based on the routes of nylon bag administration: the first group received oral administration (OS group, $n = 24$); the second group received directly at the proximal jejunum (JE group, $n = 18$); and the third group received at the proximal colonic site (CO group, $n = 18$). The OS group was divided into two subgroups of chitin ($n = 3$ each) or chitosan ($n = 9$ each). Approximately 50 mg of chitin or chitosan was packed inside each bag. The bags were immersed in water with shaking for 10 min, dried at 37°C for 7 h, and were then weighed. Three bags were administered orally to each dog. The JE and CO groups were also divided into two subgroups and received each type of chitosan ($n = 9$ each), respectively. Three bags were surgically inserted into the proximal jejunum (JE group) or the proximal colon (CO group) under general anesthesia. The bags were recovered from the feces within 2 days after administration and were washed in water for 10 min. Then the bags were dried at 38°C for 7 h and weighed.

3.3. Experiment 2

Six mongrel dogs were divided into two groups consisting of three dogs in each subgroup: colonic contents removal and no removal groups. Colon was exposed by laparotomy under general anesthesia and was ligated with 2-0 silk (Nescosuture, Azuwel Co. Ltd, Osaka, Japan) at proximal, middle, and distal sites to make two 10-cm long loops. In the group with colonic content retention, the middle site of each loop was incised longitudinally at 3 cm in length. Three nylon bags (3×1 cm^2) that contained the powder type of chitosan were inserted into the proximal loop and three similar empty bags without the chitosan particles were placed into the distal loop. In the colonic contents removal group, the colonic contents in the loops were removed with a spoon after similar incision of each loop, and then the inside of the loops was lavaged with saline at one time. Three of the similar bags with the chitosan particles were placed in the proximal loop and three bags without the chitosan particles were placed in the distal loop. The incisions in the colonic walls were closed by continuous suture with 3-0 silk (Nescosuture, Azuwel Co. Ltd, Osaka, Japan), and then the abdominal wall, subcutaneous tissue, and skin were sutured in a routine manner with 2-0 and 3-0 silk. The bags were recovered under general anesthesia at 24 h post-operatively. The bags were weighed after washing and drying in the manner described previously.

3.4. Experiment 3

Nylon bags measuring 5×5 cm^2 were made up of 300 mesh (pore size 45 μm) nylon fabric and 300 mg of chitosan (flake type) was placed into each bag. Then the bags were immersed in 300 ml of artificial gastric or

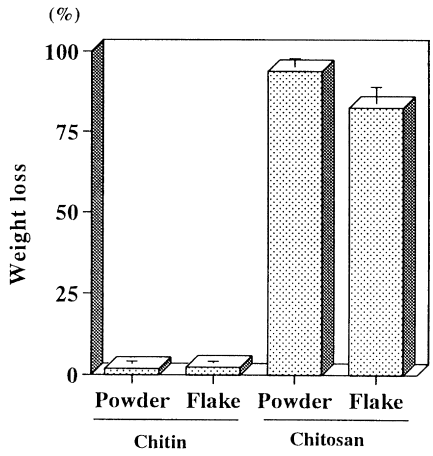

Fig. 1. Changes in weight of chitin and chitosan after oral administration.

intestinal juice and then shaken at an interval of 1 h. The bags were recovered after 1, 2, 4, 8, or 12 h and were weighed after washing and drying.

4. Statistical analysis

Statistical analysis was performed using Student's *t*-test.

5. Results

5.1. Experiment 1

The changes in the weight of chitin and chitosan in the nylon bags after oral administration are shown in Fig. 1. Chitosan with an average particle diameter of 0.5 mm (powder type) or 3 mm (flake type) lost 93.8 ± 3.9 and 82.4 ± 6.4% of its original weight, respectively; chitosan formed a film while chitin did not undergo any change in either weight or shape. There was no significant difference in weight loss between the powder and flake types in both the chitin and chitosan groups. Table 1 shows the relationship between the change in the weight of chitosan and the route of administration. When chitosan was administered into the jejunum and colon, about 40% weight loss was observed. There was no significant difference in weight loss between the JE and CO groups in each type.

Table 1
Relationship between the change in the weight of chitosan and the route of administration

Route	Shape[a]	Weight loss (%)
Oral	Powder	93.8 ± 3.9[b]
	Flake	82.4 ± 6.3
Jejunum	Powder	34.2 ± 7.5
	Flake	36.9 ± 5.6
Colon	Powder	33.8 ± 3.5
	Flake	34.4 ± 6.8

[a] Powder: 0.5 mm in diameter; Flake: 3 mm in diameter.
[b] Mean ± SD.

5.2. Experiment 2

The changes in the weight of chitosan after insertion into the colonic loops are shown in Fig. 2. When the bags containing chitosan were placed into colonic loops for 24 h, about 26% weight loss was observed with no treatment of colonic content ($p < 0.05$), while no weight loss was observed with the removal of colonic content. There was also no change in the weight of the empty bags without chitosan particles.

5.3. Experiment 3

The changes in the weight of chitosan immersed in artificial gastric and intestinal juice are shown in Fig. 3. A piece of chitosan became a gel in artificial gastric juice and its weight decreased significantly by 12% at 4 h of immersing. Almost all chitosan became a gel and its weight decreased significantly by 15% at 12 h. On the other hand, chitosan did not change in weight and shape when it was immersed in artificial intestinal juice.

6. Discussion

The present study showed that chitosan underwent physical changes in terms of weight and shape, but chitin did not, in the gastrointestinal tract when following administration in dogs. Hirano et al. (1990) fed chitin and chitosan to rabbits, hens and broilers and observed digestibility of chitin and chitosan. They reported that rabbits digested 35–85% of both chitin and chitosan, whereas it was 88–98% for hens and broilers. Nishimura et al. (1997) also suggested digestion of chitosan using ^{14}C-labeled chitosan in rats. Regarding digestibility of chitosan, our present results are consistent with their data. On the other hand, our result is not consistent with the data of Hirano et al. (1990) regarding digestion of chitin. We cannot explain clearly the difference between these results. However, some reasons are speculated. One reason is due to difference in the methods of analysis. They measured acid hydrolysis in the feces as digestibility of chitin after oral administration. We measured the weight loss of chitin in the nylon bag after oral administration. We think that measuring the loss of weight is more suitable to assay physical change of chitin as direct change is observed. Another reason could be due to species difference. They used rabbits and hens, while we used dogs. It is thought that chitinase and chitosanase digest chitin and chitosan, which are secreted from intestinal bacteria in the gastrointestinal tract. It is possible that bacterial flora is different among species.

The in vitro study also showed that chitosan became a gel in artificial gastric juice and showed only a 15% weight loss. These data are consistent with the data of Kanauchi, Deuchi,

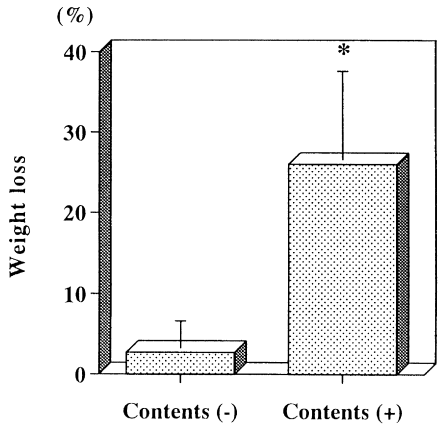

Fig. 2. Effect of colonic contents on the change in the weight of chitosan ($*: p < 0.05$ vs. original weight).

Imasato, Shizukuishi and Kobayashi (1995). From the in vivo and in vitro results, it is suggested that chitosan was lost from the bag into the gastrointestinal tract after being changed to a gel in the stomach.

In the present study, we investigated whether chitosan undergo physical changes in the small and large intestines, or not. For this experiment, we designed surgical insertion of the nylon bag containing chitosan to both intestines. From our result, chitosan was found to be influenced by the environment of the large intestine, but not by the environment of the small intestine. The present in vitro study also showed that artificial intestinal juice did not influence chitosan.

Next, we investigated the effect of colonic contents on the physical changes of chitosan because chitosan was found to be influenced by the environment of the large intestine. From the fact that 26% weight loss of chitosan was observed in the presence of colonic contents in the large intestine, it appears that bacterial flora also influenced physical changes. Hirano et al. (1990) reported that chitosanase was found in bacterial flora and in dietary fiber. The bacterium producing chitosanase is *Bacillus* spp (Izumi & Ohtakara, 1987). It is known that the main type of bacterial flora in canine feces is *Bacteroidaceae* (Tamura, 1987), but there are few data regarding other bacterial species. Accordingly, further investigation of this point should be done in the future.

To our knowledge, no one has referred at which site chitin and chitosan are digested in the gastrointestinal tract except for our previous data, which indicated that chitin was digested in the rumen of sheep, but chitosan was not (Yoshino et al., 1991). The present study clarified the site where chitosan is influenced in the gastrointestinal tract of dogs. The present result is worthy for further investigation of chitin and chitosan as health foods for human.

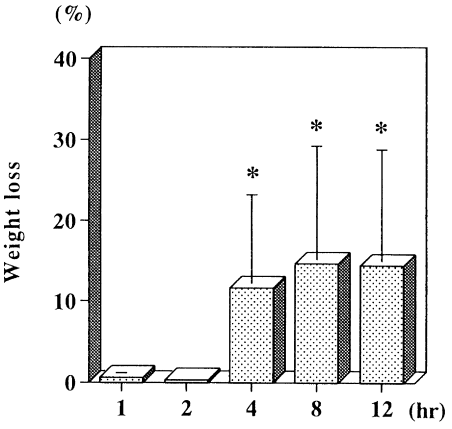

Fig. 3. Changes in the weight of chitosan in artificial gastric juice ($*: p < 0.05$ vs. original weight).

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