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ORIGINAL ARTICLE

EFFECTS OF DIETARY CHITOSAN ON NITROGEN METABOLITE LEVELS IN MICE

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Abstract

Background-Chitosan, a polymer of glucosamine, appears to interact with bile acids and/or lipids in the intestinal lumen. As it is well established that the ingestion of some types of dietary fibers has been shown to influence lipid levels, it appears possible that chitosan may also influence protein metabolism.

Methods-In the present study, we investigated the effect of chitosan on nitrogen metabolite concentrations in mice. Mice in the treatment group received 10 mg chitosan orally 2 times a day for 21 days. Serum urea and creatinine levels were measured in the test and control groups.

Results-In the chitosan-treated group, serum creatinine levels were lower as compared to controls [0.63±0.13 (mean±SD, n=90) vs. 1.14 ± 0.21 mg/dl]. A significant decrease in average serum urea level was also observed after 28 days of chitosan ingestion by the mice in the treatment group [27.45±2.53 (mean±SD, n=90) vs. 39.68±2.47 mg/dl].

Conclusion- Daily consumption of chitosan significantly reduced the serum creatinine and urea levels in mice, although there are no confirmed explanations for these effects. This work also provides an insight to the perspectives for using chitosan in clinical practice.

Keywords • Chitosan • nitrogen metabolites

Introduction

Chitosan is a polycationic polymer containing more than 5000 glucosamine units and is obtained commercially by alkaline deacetylation of chitin (a N-acetyl-glucosamine polymer) from shellfish exo-skeletons[1]. Chitosan is inexpensive, non-toxic and possesses potentially reactive amino functional groups. It has been shown to be of potential use in many different fields: as an antifungal compound in agriculture, as a flocculating agent in wastewater treatment, as a food additive in food industry, as a hydrating agent in cosmetics, and more recently, as a pharmaceutical agent in biomedicine.[2-3] It is known to have a marked hypocholesterolemic effect in cholesterol-fed rats, lowering cholesterol absorption in the lymphatic thoracic duct.[4,5]

Addition of chitosan to the basal and high fat diets of chickens decreased the apparent fat absorption ratio and abdominal fat pad weight. The increased plasma triglyceride concentration due to a high fat diet was decreased by additional chitosan.[6-8] The reduction in total cholesterol concentration and increased HDL to total cholesterol ratio was probably caused by enhanced reverse cholesterol transport in response to intestinal loss of dietary fat.[9] Chitosan effectively lowered cholesterol absorption more than guar gum or cellulose, and this effect was more significant when utilized along with fiber. Dietary fats did not modify cholesterol absorption.[10] Hypoglycemic and hypolipidemic effects of chitosan have been shown in neonatal streptozotocin-induced diabetic mice.[11] Chitosan feeding, affects the metabolism of intestinal bile acids in rats.[12] Addition of chitosan to the diet does not affect body weight gain and feed efficiency.[13] It appears possible that chitosan may also influence

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protein metabolism.

Previous studies have examined the impact of chitosan on serum nitrogen metabolite levels, although little progress has been made in the identification of these effects. In this regard, the intention of this research was to determine the effects of chitosan on serum urea and creatinine levels in mice.

Materials and Methods

Chemicals

Chitosan, kit No. 535, ferric chloride, phosphoric acid, sulphuric acid, diacetyl monoxyme and thiosemicarbazide were obtained from the Sigma Chemical Company, St. Louis, MO, USA. All solvents were of analytical grade and were tested for purity by carrying out blank runs.

Animals

The animals under study were male mice, 24-28 grams in weight and 6-8 weeks of age. They were housed six per cage and had free access to water and food. The animals were maintained in an air-conditioned room at 19-23˚C, with a 12-hour light-dark cycle. All the animals survived the study procedure without any signs of illness. Mice in the treatment group received 10 mg chitosan orally 2 times a day for 21 days. Except for the ingestion of chitosan, they all were given the same diet consisting of a commercial powder offered *ad libitum*. Each experimental period was 28 days long, the first 7 days intended for adaptation. Data was collected during the remaining 21 days. Food intake and body weight were measured daily.

Assay procedures

Blood samples for determination of serum creatinine and urea were obtained every 12 hours. Sigma kit No. 535 was used for the measurement of serum urea levels. The method was modified according to Rioux, et al.[14]

The absorbance of standard and test against blank was read at 540 nm. Absorbance versus concentration was plotted for standards and concentrations of samples and controls. The linearity of the calibration curve in duplicate with the urea calibrators was obtained.

Determination of the creatinine concentration was derived by the Jaffes caloric method in which yellow/orange coloration appears when the metabolites are treated with alkaline picrate. Sigma kit No. 555 was utilized and the method was modified according to Rioux, et al.[14] The specimens were read at 498 nm. Absorbance versus concentration was plotted for standards and concentrations of samples and controls from the curve were read.

Statistical analysis

The significance of differences between the mean of the control and test groups was determined by one-way analysis of variance followed by student's *t-test*. $P<0.05$ was considered statistically significant.

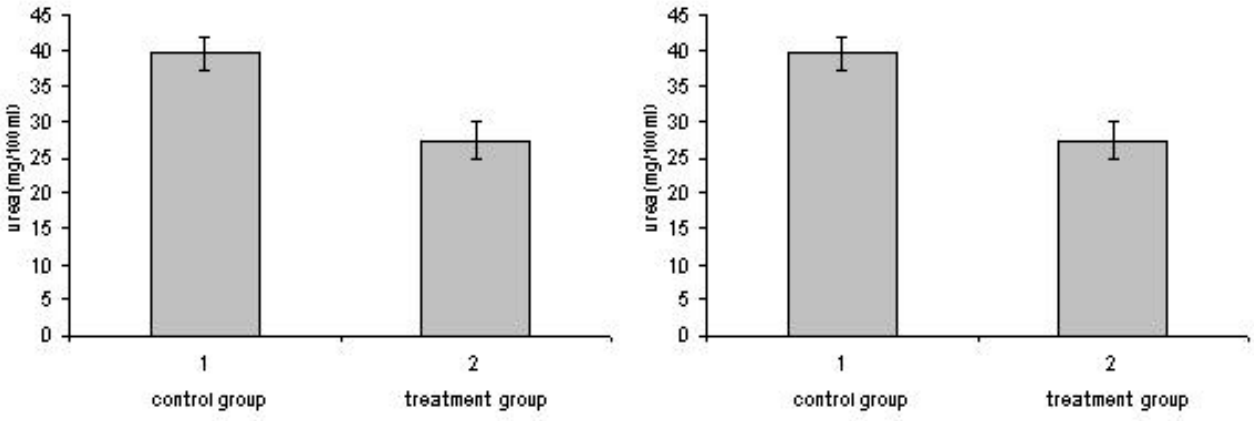

Figure 1. Effects of the chitosan on serum levels of creatinine (a) and urea (b) in mice. Each column represents the mean value ± SD in six independent experiments.

Results

The calibration curve was straight up to at least 50 mg/dl for serum urea and up to at least 1.5 mg/dl for serum creatinine. Figure (1a) shows that after ingestion of chitosan for 28 days, the average serum creatinine level of the mice in the treatment group decreased significantly as compared to that in the control group [0.63±0.13 (mean±SD, n=90) vs. 1.14±0.21 mg/dl]. Serum urea levels in the treatment group also decreased as compared to that in the control group [27.45±2.53 (mean±SD, n=90) vs. 39.68±2.47 mg/dl]. This decrease is shown in Figure 1b.

Discussion

This study has attempted to determine the effect of chitosan on nitrogen metabolites in mice. It is important to emphasize that methodological issues limit our conclusions and other biochemical parameters may produce entirely different results. Methods used in this study yield linear results for concentrations of serum creatinine and urea up to 1.2 and 50 mg/dl, respectively. Consumption of daily chitosan significantly reduced the serum creatinine and urea levels in mice. Our results are in agreement with those reported previously.[5,12] In mice, chitosan feeding causes a marked effect on the metabolism of proteins as well as lipids.[4,5,11,12]

The mode of action of chitosan on the serum level of nitrogen metabolites is not clearly understood, although possible explanations are offered. Improved renal clearance of nitrogen metabolites or combination of chitosan with the metabolites in the digestive tract and the subsequent excretion of the products in the feces are among the favored explanations.

This article highlights the importance of this novel chemical for a variety of biomedical applications. Further, this work provides an insight to the perspectives for using chitosan in clinical practice.

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