Effects of High Molecular Weight Chitosan on Neonatal Streptozotocin (STZ)-induced Noninsulin-dependent Diabetes Mellitus ICR Mice

Kyung-Yeon Eo and Oh-Deog Kwon*

College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Korea

ABSTRACT

The effect of high molecular weight (HMW, 200,000–300,000) chitosan on neonatal streptozotocin (STZ)-induced noninsulin-dependent diabetes mellitus ICR mice was investigated by analyzing body weight, food and water consumption, urine volume, non-fasting serum glucose, urine glucose, serum triglyceride, and cholesterol levels. HMW chitosan (0.2% or 0.8%) was given to the mice as drinking water for 8 weeks, and daily administration reduced body weight, water consumption, urine volume, serum triglyceride and cholesterol levels; however, HMW chitosan had no effect on blood and urine glucose levels in these mice.

Key words: diabetes, high molecular weight chitosan, streptozotocin, mice.

INTRODUCTION

Diabetes mellitus is one of the most common endocrinopathies (1) and a heterogeneous condition characterized by a relative or absolute deficiency of insulin secretion by the beta cells of the islets of Langerhans in the pancreas in humans and animals (2). Diabetes mellitus is classified into two types: type 1 (insulin-dependent diabetes mellitus, IDDM) and type 2 (non-insulin dependent diabetes mellitus, NIDDM) (3). NIDDM arises from a combination of insulin resistance and abnormal insulin secretion (4) and is a diabetic state in which insulin secretion is usually sufficient to prevent ketosis but not adequate to prevent hyperglycemia and overcome insulin resistance in peripheral tissues (5).

Several investigators have reported the antidiabetic actions of various plants including *Scoparia dulcis* (6), *Casearia esculenta* (7), and *Astragalus* saponine I (8), and much research has examined the antidiabetic effects of chitosan (3,9). However, previous studies have mainly focused on low molecular weight (LMW, ≤ 50,000) chitosan or chitooligosaccharides due to their shorter chain (10,11). Although LMW chitosan is easily soluble in water and absorbed through the body, the chemical or enzymatic procedures required to make LMW chitosan have some limits (12,13). Chemical hydrolysis is likely to produce some toxic compounds, environmental pollution, and lower production yield (12). Enzymatic hydrolysis lacks proper technology to produce the desired molecular weights from large-scale production (12). Furthermore, all the procedures are quite expensive (12).

While high molecular weight (HMW, ≥ 200,000) chitosan has poor solubility and absorbability (12,13), it is interesting to examine its biological effects on diabetes. Therefore, we tested the antidiabetic effects of HMW chitosan on neonatal STZ-induced NIDDM ICR mice by analyzing body weight, food and water consumption, urine volume, non-fasting serum glucose, serum triglyceride, and cholesterol levels.

MATERIALS AND METHODS

Compounds

Streptozotocin (STZ) was obtained from Sigma (St. Louis, MO, USA). HMW chitosan (MW: 200,000 - 300,000) was kindly provided by Jakwang Co., Ltd. (Seoul, Korea).

Animals and treatment

Healthy male ICR mice were obtained from the Orient Company (Seoul, Korea) and kept for breeding. The animals were housed in polypropylene cages with steel grid tops and allowed free access to a dry pellet diet (standard rodent chow 5057; Purina Korea, Seoul, Korea) and tap water. The animal rooms were controlled at 23 ± 2°C, 55 ± 5% humidity, a ventilation frequency exceeding 12 changes/h, and a 12-h light/dark cycle.

STZ was freshly dissolved in 0.05 M sodium citrate buffer (pH 4.5) and administered within 10 min of dissolution. NIDDM was induced by intraperitoneal injection of STZ (80 mg/kg) to 3-day-old neonatal pups (11). The experiments were conducted 7 weeks after the STZ injection. Mice with serum glucose levels above 400 mg/dl were considered diabetic (14).

Experimental procedure

HMW chitosan (0.2 and 0.8% dissolved in distilled water)
was given to diabetic mice as drinking water for 8 weeks. Other STZ mice were given tap water alone. Blood samples were obtained from the cavenous sinus through a capillary at 0, 4, and 8 weeks after treatment to determine serum chemistry levels. The body weight of each mouse was measured immediately before blood collection. After blood collection, the animals were kept in individual metabolic cages for 24 h. Food consumption, drinking water, and urine volume per 24 h were measured from individual animals. Separated serum and urine samples were stored at \(-80^\circ\text{C}\) until the measurements were made.

**Serum chemistry and urine glucose analysis**

Serum and urine glucose levels were determined using an automated chemistry analyzer (HITACHI 710, Hitachi, Japan). Serum triglyceride and cholesterol levels were determined using an automated chemistry analyzer (COBAS INTEGRA 700, Roche, Switzerland).

**Statistical analysis**

All the data are expressed as mean \(\pm\) S.D. Statistical significances were determined by one-way ANOVA. A \(p<0.05\) was considered statistically significant.

**RESULTS**

**Food consumption and body weight**

The average food consumption in the STZ group was 7.60 \(\pm\) 0.90 g at day 0, 9.70 \(\pm\) 1.10 g at 4 wk, and 6.12 \(\pm\) 0.80 g at 8 wk. In the STZ + 0.2% chitosan group, it was 7.80 \(\pm\) 0.80 g at day 0, 8.20 \(\pm\) 0.90 g at 4 wk, and 6.39 \(\pm\) 0.70 g at 8 wk. In the STZ + 0.8% chitosan group, it was 9.20 \(\pm\) 1.30 g at day 0, 8.60 \(\pm\) 0.20 g at 4 wk, and 7.28 \(\pm\) 0.90 g at 8 wk. Differences in food consumption among the groups were not significant.

The average body weights in the STZ group were 30.9 \(\pm\) 2.47 g at day 0, 37.7 \(\pm\) 3.64 g at 4 wk, and 39.4 \(\pm\) 3.88 g at 8 wk. The average body weights in the STZ + 0.2% chitosan group were 32.1 \(\pm\) 1.91 g at day 0, 35.4 \(\pm\) 1.13 g at 4 wk, and 33.7 \(\pm\) 7.21 g at 8 wk; these weights tended to be lower than those in the STZ group, but differences among the groups were not significant. However, the average body weights in the STZ + 0.8% chitosan group (29.2 \(\pm\) 3.38 g at day 0, 31.8 \(\pm\) 2.36 g at 4 wk \([p<0.05]\), and 31.2 \(\pm\) 0.48 g at 8 wk \([p<0.05]\)) were significantly lower than those in the STZ group (Fig. 1).

**Water consumption and urine volume**

The water consumption per 24 h in the STZ group was 50.0 \(\pm\) 3.8 ml at day 0, 60.0 \(\pm\) 4.5 ml at 4 wk, and 56.7 \(\pm\) 3.7 ml at 8 wk. The water consumption per 24 h in the STZ + 0.2% chitosan group (51.0 \(\pm\) 2.1 ml at day 0, 48.8 \(\pm\) 2.4 ml at 4 wk \([p<0.01]\), and 45.0 \(\pm\) 1.6 ml at 8 wk \([p<0.01]\)) was significantly lower than that in the STZ group. The water consumption per 24 h in the STZ + 0.8% chitosan group (52.0 \(\pm\) 4.6 ml at day 0, 45.0 \(\pm\) 3.6 ml at 4 wk \([p<0.01]\), and 40.0 \(\pm\) 1.9 ml at 8 wk \([p<0.01]\)) was significantly lower than that in the STZ group (Fig. 2).

The urine volumes per 24 h in the STZ group were 45.4 \(\pm\) 5.2 ml at day 0, 53.3 \(\pm\) 3.8 ml at 4 wk, and 51.7 \(\pm\) 4.6 ml at 8 wk. The urine volumes per 24 h in the STZ + 0.2% chitosan group (42.8 \(\pm\) 6.4 ml at day 0, 46.3 \(\pm\) 1.8 ml at 4 wk \([p<0.01]\), and 41.5 \(\pm\) 3.1 ml at 8 wk \([p<0.01]\)) were significantly lower than those in the STZ group. The urine volumes per 24 h in the