J Vet Clin 25(6) : 452-457 (2008)

Journal of Veterinary Clinics
www.ksvc.or.kr

Effects of Chitosan on Kidney Function in Streptozotocin-Induced Diabetic Mice

Kwang-Won Sun and Oh-Deog Kwon[1]

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

(Accepted: October 02, 2008)

Abstract : This study examined the effects of the daily administration of high-molecular-weight (HMW) chitosan in drinking water (0.8 %) on kidney function in streptozotocin (STZ)-induced diabetic ICR mice. The HMW chitosan lowered the blood urea nitrogen (BUN), serum and urine creatinine levels, urine protein levels, and albuminuria and reduced the kidney weight in STZ-induced type 1 diabetic ICR mice. On histopathological findings, capillary loops are open, but narrowed, and the mesangial matrix enlarged in the STZ-induced diabetic ICR mice. By contrast, the capillary loops and mesangial matrix of the chitosan-treated ICR mice were nearly normal compared with the STZ-induced diabetic ICR mice.

Key words **:** chitosan, diabetes mellitus, kidney, mouse.

Introduction

Insulin-dependent diabetes mellitus (IDDM), also referred to as type 1 diabetes, is a diabetic state in which endogenous insulin deficiency or impaired insulin action results in carbohydrate intolerance, and abnormal protein and lipid metabolism (3). With a relative or absolute deficiency in insulin, the glucose obtained from the diet or via hepatic gluconeogenesis is not utilized efficiently or normally by muscle adipose tissue or the liver itself (2). Consequently, glucose accumulates in the blood (hyperglycemia) and when its concentration exceeds the renal tubular maximum transport threshold, it spills into the urine (glucosuria) (4). Glucosuria creates an osmotic diuresis, causing polyuria and obligatory polydipsia (2,4,18). Diabetic nephropathy (DN) caused by the osmotic diuresis is a major complication of diabetic mellitus and is one of the main causes of death in humans, dogs, and cats (2-4,14,24).

Several investigators have reported the antidiabetic actions of various materials. The root extract of *Casearia esculenta* has an antihyperglycemic effect and may alleviate the liver and renal damage in streptozotocin (STZ)-induced diabetic rats (22). The effects of *Astragalus* saponin I (AS I), a component extracted from *Astragalus membranaceus*, on the diabetic nephropathy induced by the administration of STZ to male rats was studied (30). An aqueous extract of *Scoparia dulcis* had an antidiabetic effect in STZ-induced diabetic male rats (21).

Chemically, chitosan is a polymeric D-glucosamine, a basic polysaccharide, and is prepared by the alkaline deacetylation of chitin derived from the shells of crabs, shrimp, shellfish, and insects (16). It is a hydrophilic, biocompatible, and biodegradable polymer with low toxicity (11). Much research has examined the biological activities of chitosan, such as its immunopotentiating (15), wound healing (19), cholesterol lowing (20,26), antibacterial (27), anti-ulcer (10), and antidiabetic (7,13) effects. However, the effects of chitosan on kidney function in STZ-induced diabetic mice are not clear. Therefore, this study investigated the effects of high-molecular-weight (HMW) chitosan on kidney function in STZ-induced diabetic mice.

Materials and Methods

Compounds and chemicals

Streptozotocin was obtained from Sigma Chemical (St. Louis, MO). Chitosan (molecular weight 200,000–300,000) was kindly provided by Jakwang (Seoul, Korea).

Animal treatment

Male ICR mice were obtained at 4 weeks of age from Orient Company (Seoul, Korea) and acclimated for 1 week. 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 throughout the acclimatization and experimental periods. 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.

To induce type I diabetes, STZ was freshly dissolved in 0.05 M sodium citrate buffer (pH 4.5) and administered

[1]Corresponding author.
E-mail : odkwon@knu.ac.kr

within 10 min of dissolution. Type I diabetes was induced by a single intraperitoneal injection of STZ (200 mg/kg) (5). Control animals were injected with vehicle (sodium citrate buffer). The experiments were conducted 1 week after the injection of STZ or vehicle. Mice with serum glucose levels above 400 mg/dl were considered diabetic (25).

Treated mice were injected subcutaneously with long-acting, heat-treated ultralente insulin (Novo Nordisk A/S, Bagsvaerd, Denmark) at a dose of 8 IU/kg daily.

Experimental procedures

Chitosan (0.8 %) was given to the mice in drinking water for 8 weeks beginning at 6 weeks of age. Control mice were given tap water instead of chitosan solution. Blood samples were withdrawn from the cavernous sinus through a capillary tube at 0, 4, and 8 weeks after starting treatment to determine serum chemistry levels. After collecting the blood samples, these animals were kept in individual metabolic cages, and the 24-h urine volume was measured. Separated serum and urine were stored at -80 °C until the measurements were made. After treatment for 8 weeks, the animals were killed, and the kidneys were removed, cleaned carefully, and weighed.

Blood and urine chemistry

Serum creatinine was analyzed using the Jaffe method with a Cobas Integra 700 (Roche). Blood urea nitrogen (BUN) was analyzed using a kinetic test with urease and glutamate dehydrogenase (GLDH) with a Cobas Integra 700 (Roche). Urine creatinine was measured with the Jaffe method with a Hitachi 7180 (Hitachi). Urine protein was determined using the benzethonium chloride method with a Hitachi 7180 (Hitachi). Urine microalbumin was analyzed using a turbidimetric method with a Hitachi 7180 (Hitachi).

Histopathology

The kidneys were fixed in 10 % neutral formalin, and then embedded in paraffin, sectioned (4 μm), and stained with hematoxylin and eosin (H&E). The changes in the glomeruli in each group were observed under a light microscope (Axioskop 40; Carl Zeiss, Oberkochen, Germany).

Statistical analysis

All data were expressed as the mean ± SD. Statistical analysis consisted of the *t*-test. In all cases, $p<0.05$ was considered significant.

Results

BUN

Fig 1. shows the effects of chitosan in drinking water given to STZ-induced diabetic ICR mice on the BUN levels after treatment for 8 weeks. The BUN levels of the normal control mice were 23.4–26.8 mg/dl throughout the 8-week observation period beginning at 6 weeks of age. The BUN levels of the STZ group were significantly higher than those of the control group after treatment for 8 weeks [STZ vs. control: 4 weeks, 39.7 ± 9.00 vs. 26.8 ± 6.10 mg/dl ($p<0.01$); 8 weeks, 38.7 ± 12.71 vs. 23.4 ± 3.47 mg/dl ($p<0.01$)]. The BUN levels of the STZ+chitosan group were lower than those of the STZ group, although the difference was not significant. In addition, the BUN levels of the STZ+insulin and STZ+insulin+chitosan groups were lower than those of the STZ group ($p<0.05$ and $p<0.01$, respectively).

Serum creatinine

Fig 2. shows the effects of giving chitosan in drinking water to STZ-induced diabetic ICR mice on the serum creatinine levels after treatment for 8 weeks. The serum creatinine levels of the normal control mice were 0.125–0.150 mg/dl throughout the 8-week observation period beginning at 6 weeks of age. The serum creatinine levels of the STZ group

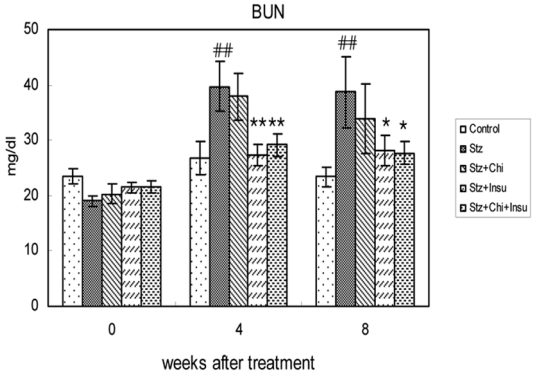

Fig 1. The effect of chitosan given in drinking water for 8 weeks on the BUN in STZ-induced diabetic ICR mice. Each plot denotes the mean ± SD for 8 mice. *$p<0.05$, **$p<0.01$: Significantly different from the respective STZ mice. ##$p<0.01$: Significantly different from the respective control mice.

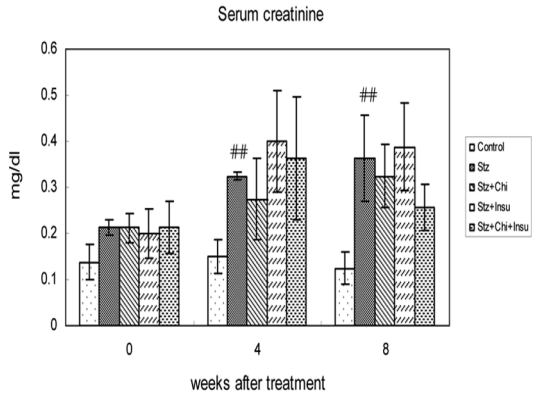

Fig 2. The effect of chitosan given in drinking water for 8 weeks on the serum creatinine in STZ-induced diabetic ICR mice. Each plot denotes the mean ± SD for 8 mice. ##$p<0.01$: Significantly different from the respective control mice.

were significantly higher than those of the control group after treatment for 8 weeks [STZ vs. control: 4 weeks, 0.325 ± 0.104 vs. 0.150 ± 0.076 mg/dl (p<0.01); 8 weeks, 0.363 ± 0.185 vs. 0.125 ± 0.071 mg/dl (p<0.01)]. Chitosan lowered the serum creatinine levels of the STZ-induced diabetic ICR mice after treatment for 8 weeks [STZ+chitosan vs. STZ: 4 weeks, 0.275 ± 0.175 vs. 0.325 ± 0.104 mg/dl; 8 weeks, 0.325 ± 0.139 vs. 0.363 ± 0.185 mg/dl], although the difference was not significant. In addition, the serum creatinine levels of the STZ+insulin+chitosan group were lower than those of the STZ+insulin group after treatment for 8 weeks, but again the difference was not significant.

Urine creatinine

Fig 3. shows the effects of chitosan in drinking water given to STZ-induced diabetic ICR mice on the urine creatinine levels after treatment for 8 weeks. The urine creatinine levels of the normal control mice were 0.29–0.37 mg/24h throughout the 8-week observation period beginning at 6 weeks of age. The urine creatinine levels of the STZ group were significantly higher than those of the control group and increased gradually with time over the 8 weeks of treatment [STZ vs. control: 1.51 ± 0.38 vs. 0.29 ± 0.08 mg/24h, 1.69 ± 0.19 vs. 0.37 ± 0.28 mg/24h, 1.93 ± 0.56 vs. 0.36 ± 0.35 mg/24h at day 0 (p<0.05), 4 (p<0.05), and 8 (p<0.05) weeks, respectively]. Chitosan effectively lowered the urine creatinine levels of the STZ-induced diabetic ICR mice after treatment for 8 weeks [STZ+chitosan vs. STZ: 4 weeks, 1.32 ± 0.27 vs. 1.69 ± 0.19 mg/24h, 8 weeks, 1.31 ± 0.41 vs. 1.93 ± 0.56 mg/24h, p<0.05, respectively]. However, the urine creatinine levels of the STZ+insulin group increased unexpectedly after 8 weeks of treatment.

Urine protein

Fig 4. shows the effects of chitosan in drinking water given to STZ-induced diabetic ICR mice on the urine protein levels after treatment for 8 weeks. The urine protein levels of the normal control mice were 7.85–10.02 mg/24h throughout the 8-week observation period beginning at 6 weeks of age. The urine protein levels of the STZ group were significantly higher than those of the control group after treatment for 8 weeks [STZ vs. control: 110.69 ± 43.01 vs. 7.85 ± 4.06 mg/24h, 178.55 ± 12.66 vs. 9.18 ± 7.39 mg/24h, 144.65 ± 21.43 vs. 10.02 ± 9.98 mg/24h at day 0 (p<0.01), 4 (p<0.01), and 8 (p<0.01) weeks, respectively]. Chitosan effectively lowered the urine protein levels of the STZ-induced diabetic ICR mice after treatment for 8 weeks [STZ+chitosan vs. STZ: 4 weeks, 92.10 ± 6.08 vs. 178.55 ± 12.66 mg/24h, 8 weeks, 113.40 ± 12.55 vs. 144.65 ± 21.43 mg/24h, p<0.05, respectively]. The urine protein levels of the STZ+insulin and STZ+insulin+chitosan groups were lower than those of the STZ group (p<0.05 and p<0.01, respectively). In addition, the urine protein levels of the STZ+insulin+chitosan group were lower than those of the STZ+insulin group [STZ+insulin+chitosan vs. STZ+insulin: 4 weeks, 38.30 ± 7.42 vs. 95.60 ± 56.52 mg/24h, 8 weeks, 49.20 ± 4.30 vs. 64.00 ± 7.97 mg/24h, both p<0.05].

Albuminuria

Fig 5. shows the effects of chitosan in drinking water given to STZ-induced diabetic ICR mice on the urine albumin/creatinine ratio after treatment for 8 weeks. The urine albumin/creatinine ratio of the normal control mice was 0.008–0.020 throughout the 8-week observation period beginning at 6 weeks of age. Albumin excretion was increased in the STZ-induced diabetic mice [STZ vs. control (albumin/creatinine ratio): 0.104 ± 0.046 vs. 0.020 ± 0.008, 0.153 ± 0.041 vs. 0.012 ± 0.013, 0.154 ± 0.051 vs. 0.008 ± 0.002 at day 0 (p<0.05), 4 (p<0.05), and 8 (p<0.05) weeks, respectively]. Chitosan effectively lowered the albumin excretion of the STZ-induced diabetic ICR mice after treatment

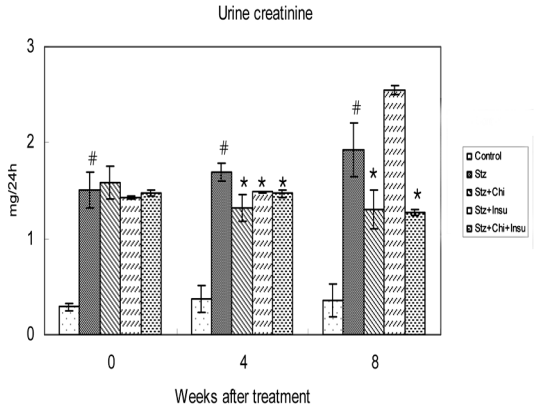

Fig 3. The effect of chitosan given in drinking water for 8 weeks on the urine creatinine in STZ-induced diabetic ICR mice. Each plot denotes the mean ± SD for 8 mice. *p<0.05: Significantly different from the respective STZ mice. $^{\#}p$<0.05: Significantly different from the respective control mice.

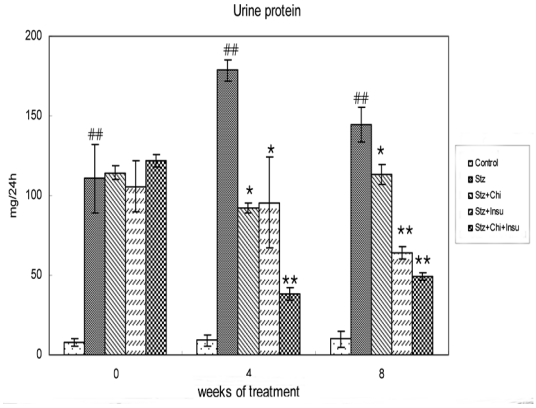

Fig 4. The effect of chitosan given in drinking water for 8 weeks on urine protein in STZ-induced diabetic ICR mice. Each plot denotes the mean ± SD for 8 mice. *p<0.05, **p<0.01: Significantly different from the respective STZ mice. $^{\#\#}p$<0.01: Significantly different from the respective control mice.

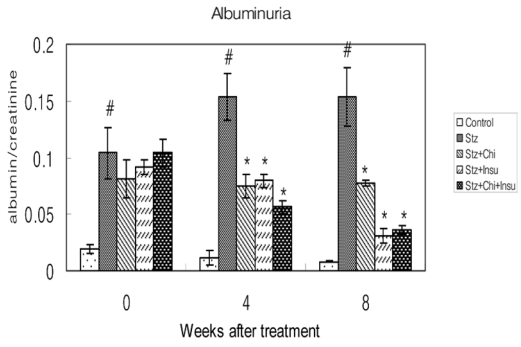

Fig 5. The effect of chitosan given in drinking water for 8 weeks on albumin excretion in STZ-induced diabetic ICR mice. Each plot denotes the mean ± SD for 8 mice. *p<0.05: Significantly different from the respective STZ mice. $^{\#}p$<0.05: Significantly different from the respective control mice.

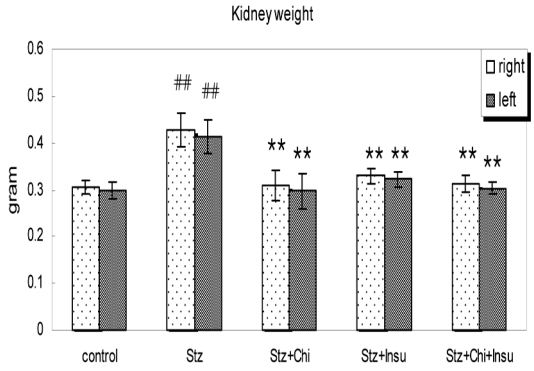

Fig 6. The effect of chitosan given in drinking water for 8 weeks on kidney weight in STZ-induced diabetic ICR mice. Each plot denotes the mean ± SD for 8 mice. **p<0.01: Significantly different from the respective STZ mice. $^{\#\#}p$<0.01: Significantly different from the respective control mice.

for 8 weeks [STZ+chitosan vs. STZ (albumin/creatinine ratio): 4 weeks, 0.075 ± 0.021 vs. 0.153 ± 0.041, 8 weeks, 0.077 ± 0.006 vs. 0.154 ± 0.051, p<0.05, respectively]. In addition, the albumin excretion of the STZ+insulin and STZ+insulin+chitosan groups was lower than that of the STZ group (both p<0.05).

Kidney weight

Fig 6. shows the effects of giving chitosan in drinking water to STZ-induced diabetic ICR mice on the weight of the kidneys of animals killed after 8 weeks of treatment. The kidneys of the normal control mice weighed 0.307 ± 0.029 g (right) and 0.298 ± 0.035 g (left). The kidneys of the STZ group were significantly heavier than those of the control group [right, 0.428 ± 0.072 g; left, 0.414 ± 0.070 g; both p<0.01]. Chitosan effectively decreased the kidney weight of the STZ-induced diabetic ICR mice [STZ+chitosan vs. STZ: right, 0.309 ± 0.065 vs. 0.428 ± 0.072 g (p<0.01); left, 0.297 ± 0.074 vs. 0.414 ± 0.070 g (p<0.01)]. In addition, the kidneys of the STZ+insulin+chitosan group were lighter than those of the STZ+insulin group [STZ+insulin+chitosan vs. STZ+insulin: right, 0.311 ± 0.036 vs. 0.329 ± 0.030 g; left, 0.303 ± 0.024 vs. 0.323 ± 0.033], but the difference was not significant.

Histopathological observation

The glomerulus capillary loops were open, and the glomeruli were relatively small in the control mice. Glomerular hypertrophy of the proximal convoluted tubules, dilated distal tubules, sloughed glomerular structure of epithelial cells, increased mesangial matrix, narrowed lumen of the capillary loops, and reduced glomerulosclerosis were observed in the STZ-induced diabetic ICR mice. By contrast, the capillary loops and mesangial matrix of the chitosan-treated ICR mice were nearly normal (Fig. 7A-C) compared with the STZ-induced diabetic ICR mice, although regional protein casting was noted in the tubules.

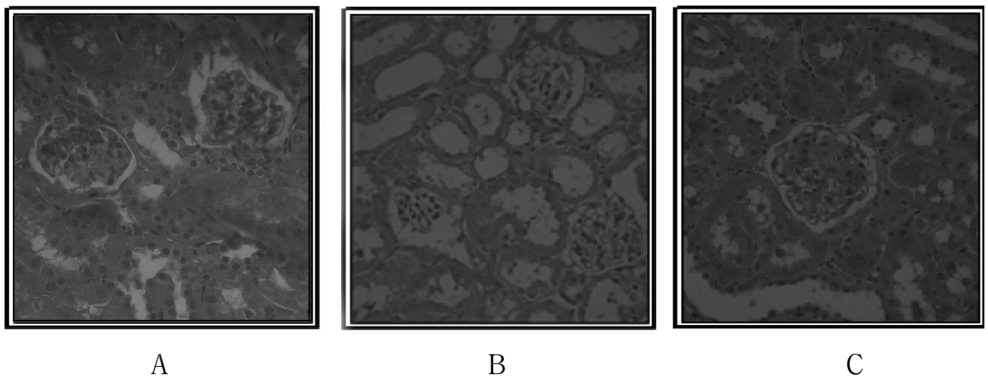

Fig 7. Light microphotographs of the kidney (H&E stain, ×40). A: control mice. The glomerulus capillary loops are open and the glomeruli are relatively small. B: STZ-treated mice. The capillary loops are open, but narrowed, and the mesangial matrix enlarged. C: Chitosan-treated mice. The glomeruli are essentially normal.

Discussion

Diabetic nephropathy (DN) is the most common cause of end-stage renal failure in developed countries and its incidence continues to rise (24). The pathologic changes of DN have been well defined in insulin-dependent diabetes mellitus (IDDM) (14).

Imaeda *et al*. (9) and Maeda *et al*. (14) reported that the plasma creatinine and BUN concentrations increase gradually after the onset of diabetes. In our study, the BUN and serum creatinine of STZ-induced diabetic mice were increased. However, the daily administration of HMW chitosan in drinking water reduced the BUN and serum creatinine in STZ-induced diabetic ICR mice. Banes *et al*. (1) reported that the creatinine excretion was increased significantly in STZ-treated rats. In our study, the urine creatinine of STZ-induced diabetic mice was increased compared with the control group beginning 1 week after STZ treatment. However, the urine creatinine of the STZ-induced diabetic ICR mice decreased with the administration of HMW chitosan. Kwak *et al*. (12) reported that urinary protein was increased in STZ-induced diabetic rats. In this study, the urinary protein of STZ-induced diabetic mice was increased significantly compared with control mice beginning 1 week after STZ treatment, and HMW chitosan effectively decreased the urine protein levels. Hartner *et al*. (6) reported that albumin excretion was increased in STZ-treated diabetic rats. The urinary microalbumin (mAlb) level is indicative of early and mild kidney injury (17). In our experiment, the albumin excretion of STZ-induced diabetic mice was increased significantly compared with control mice beginning 1 week after STZ treatment. HMW chitosan effectively decreased the albumin excretion. These results indicate that urinary creatinine, protein, and the albumin/creatinine ratio are useful for detecting early kidney injury.

Maeda *et al*. (14) reported that the kidney weight increased after the onset of diabetes and reached a plateau by day 20 in the nonobese diabetic mouse. And, Hartner *et al*. (6) reported that the kidney weight was increased in the STZ-induced diabetic rat. In our study, the kidney weight of STZ-induced diabetic mice were increased compared with control mice, and HMW chitosan effectively reduced the kidney weight.

Volkmann and Wehner (28) reported that the dilatation of small intrarenal arteries and arterioles in diabetic mice may result from progressive impairment of vasoconstriction and may be a cause of the glomerular hyperfiltration that occurs in diabetes. Reddi *et al*. (23) also reported mesangial matrix accumulation in KK mice. Huang and Preisig (8), Wolf and Ziyadeh (29), and Maeda *et al*. (14) demonstrated that the glomerular diameter was increased at the early stage of diabetes. Hartner *et al*. (6) reported macrophage infiltration and matrix expansion in the diabetic rat kidney. Yin *et al*. (30) reported that *Astragalus* saponin I (AS I), a component extracted from *Astragalus membranaceus*, decreased the thickness of the glomerular basal membrane in the early stage of diabetic nephropathy caused by the administration of STZ to male rats. However, the effects of chitosan on the histopathological changes in the kidneys of diabetic mice were not well defined. In our study, the glomerular capillary loops of control mice were open, and the glomeruli were relatively small. By contrast, glomerular hypertrophy and an increase in the mesangial matrix were observed in STZ-induced diabetes ICR mice. The mesangial matrix expansion narrowed the lumen of the intercapillary loops and reduced glomerulosclerosis. By contrast, the capillary loops and mesangial matrix of the kidney were improved in the chitosan-treated mice. The results of this experiment suggest that HMW chitosan is a potential therapeutic agent for preventing the kidney dysfunction of type 1 diabetes mellitus (IDDM).

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