PROTECTIVE EFFECT OF OLIGOCHITOSAN ON CCL_4—INDUCED ACUTE LIVER INJURY IN MICE

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Abstract

Objective To investigate the protective effects of oligochitosan on chemical hepatic injury induced by carbon tetrachloride (CCl_4) in mice. **Methods** Liver injury model was established by administration of CCl_4 into mice. The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the contents of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) in hepatic tissues were measured. The hepatic histological changes were observed by optical microscope. **Results** Oligochitosan (given at 167 and 500 mg/kg) remarkably inhibited the rises of serum ALT and AST, and decreased the content of MDA in hepatic tissues, meanwhile increased the activities of SOD in hepatic tissues. Furthermore, the pathological changes were also significantly improved in the mice of treated oligochitosan groups. **Conclusion** Oligochitosan had protective effects on the acute hepatic injury induced by CCl_4 in mice.

Key words oligochitosan; carbon tetrachloride; hepatoprotective

Introduction

Chitosan is a partially deacetylated polymer of N-acetyl glucosamine, which is obtained from chitin. Oligochitosan (COS), partially hydrolyzed products of chitosan is of great interest in medical and pharmaceutical not only for their nontoxicity and high solubility, but for the characteristics of biological functions such as antimicrobial activity [1], antioxidant ability [2], antitumor activity [3], and immuno-enhancing effects [4].

Among various chemicals that specifically injure the liver, carbon tetrachloride (CCl_4) is an extensively used xenobiotic to induce lipid peroxidation and toxicity. CCl_4 is metabolized by cytochrome P4502E1 (CYP2E1) to the trichloromethyl radical (CCl_3•), which is assumed to initiate free radical mediated lipid peroxidation leading to the accumulation of lipid-derived oxidation products (malondialdehyde) that cause liver injury[5].

The experiment was designed to investigate the antioxidative and hepatoprotective effects of oligochitosan against CCl_4 induced liver toxicity in mice by measuring the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the contents of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) in hepatic tissues, meanwhile the hepatic histological changes were observed by optical microscope.

Material and Methods

Animals and materials

Forty-eight male mice of kunming strain (weighting 22±3g) were supplied by the Animal Center of Dalian Medical University

Oligochitosan was prepared from enzymatic hydrolysis of chitosan (the degree of N-acetylation was below 5%) and separated with membrane [6].Biphenyl dimethyl dicarboxylate pilules (DDB) and CCl_4 were obtained from Beijing Union Pharmaceutical Factory. Detection kits AST, ALT, MDA,

SOD were purchased from Nanjing Jiancheng bioengineering Institute (Nanjing, P. R. China). All other chemicals were of high purity from commercial sources.

Model establishment and treatment

Mice were housed and fed a standard diet ad libitum for one-week adaptation period. After that, the animals were divided randomly into six groups of eight mice each, including normal group, model group, positive control group and groups treated with oligochitosan 50, 167, 500 mg/kg. Normal and model groups were treated with 0.9% NaCl, the others were intragastrically (ig) administrated with various concentrations of oligochitosan or the positive drug DDB 200 mg/kg, once a day for 7d. After the last drug administration 1h, except for the normal group, the others were all intraperitoneally (ip) injected with 0.2% CCl_4 corn oil solution 10 ml/kg to establish experimental model of acute liver damage. Mice were humanely killed 24h after the treatment with CCl_4 and their blood was collected. Serum was separated by centrifugation, and serum AST and ALT activities were estimated spectrophotometrically using kits. Liver tissue slices from each mouse were prepared for pathology evaluation. Meanwhile liver homogenates were to analyze liver lipid peroxidation levels by measuring MDA using kit, and total SOD activity was determined also by kit.

Statistical analysis

All data were expressed as mean±SD. Data were assessed by using *t* test, and $P<0.05$ was considered statistically significant.

Results and Discussion

Effects of oligochitosan on serum ALT and AST of mice with acute liver damage induced by CCl_4

Oligochitosan decreased the high serum ALT and AST level induced by the administration of CCl_4, as well as the cellular damage of liver (Table 1).

Table 1 Effects of oligochitosan on serum ALT and AST of mice with acute liver damage induced by CCl_4 (mean±SD)

Groups	Mice(n)	ALT(IU/L)	AST(IU/L)
Normal	8	22.90±3.29**	38.00±5.98**
Model control	8	4059.13±661.60	1928.00±563.66
COS(50 mg/kg)	8	2861.88±1404.35*	1413.47±922.53
COS(167 mg/kg)	8	2790.78±947.50**	1048.35±427.73**
COS(500 mg/kg)	8	2270.22±864.31**	1116.31±429.12**
Positive control	8	1795.45±1031.81**	1207.41±519.69*

* $P<0.05$, ** $P<0.01$ vs model control

Effects of oligochitosan on MDA and SOD of mice with acute liver damage induced by CCl_4.

Oligochitosan could lower the increase of MDA resulted from CCl_4, and the SOD was increased in groups treated with different concentrations of oligochitosan in a dose-dependent manner compared with model group (Table 2).

Table 2 Effects of oligochitosan on MDA and SOD of mice with acute liver damage induced by CCl_4 (mean±SD)

Groups	Mice(n)	MDA(nmol/mgprot)	SOD(U/mgprot)
Normal	8	5.44±1.32**	232.93±53.86**
Model control	8	20.63±3.30	165.28±34.79
COS(50 mg/kg)	8	10.47±1.09**	171.86±99.32
COS(167 mg/kg)	8	10.42±2.93**	266.96±88.58**
COS(500 mg/kg)	8	10.05±2.77**	541.96±28.66**
Positive control (DDB)	8	10.22±4.09**	581.60±31.95**

* $P<0.05$, ** $P<0.01$ vs model control

The histological changes associated with the hepatoprotective activity in three groups of oligochitosan basically supported the estimation of the serum enzyme activities. The livers of

CCl_4-treated mice showed a massive necrosis, fatty change, broad infiltration of the lymphocytes and Kupffer cells around the central vein and loss of cellular boundary [Fig.1(B)]. The histological pattern of the livers of the mice treated with oligochitosan showed marked improvement over CCl_4 control group [Fig.1(C)-(E)].

The elevated levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were presumptive markers of liver injury [7]. Enhanced susceptibility of hepatocytes cell membrane to the CCl_4-induced peroxidative damage might have resulted in increased release of the diagnostic marker enzymes into the blood circulation. In the present study, co-administration of oligochitosan evidently decreased the levels of diagnostic marker enzymes in serum as compared to the group only given CCl_4, which was in line with the results of the liver tissue slices. These results indicated the hepatoprotective action of oligochitosan.

Recently, Jeon T.I. *et al* [8] have reported that chitosan (MW 380000) protected rats from chronic CCl_4-induced lipid peroxidation. Shon Y.H. *et al* [9] also have proved that a higher-chitooligosaccharide mixture (3000<MW<5000) could be against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced lipid peroxidation. In our study, the values of the sum of MDA decreased significantly following oligochitosan administration (Table 2), which was in line with before study. Jae-Young Je *et al* [10] have ascertained hetero-chitooligosaccharides have radical scavenging activity and antioxidant activity *in vitro*. In our study, we found that oligochitosan had an antioxidant effect on acute CCl_4 induced hepatic injury. Moreover, the antioxidant enzyme (SOD) activities were increased by oligochitosan (Table 2). These results showed that the antioxidant action of oligochitosan significantly reduced the damage of CCl_4 induced liver injury and activated the biological defense system of the liver.

In conclusion our results indicated that oligochitosan given orally was able to protect mice from CCl_4-induced acute hepatotoxicity, most probably by its antioxidant activity. More studies are needed to ascertain what molecular mechanism is responsible for the hepatoprotective effect of oligochitosan.

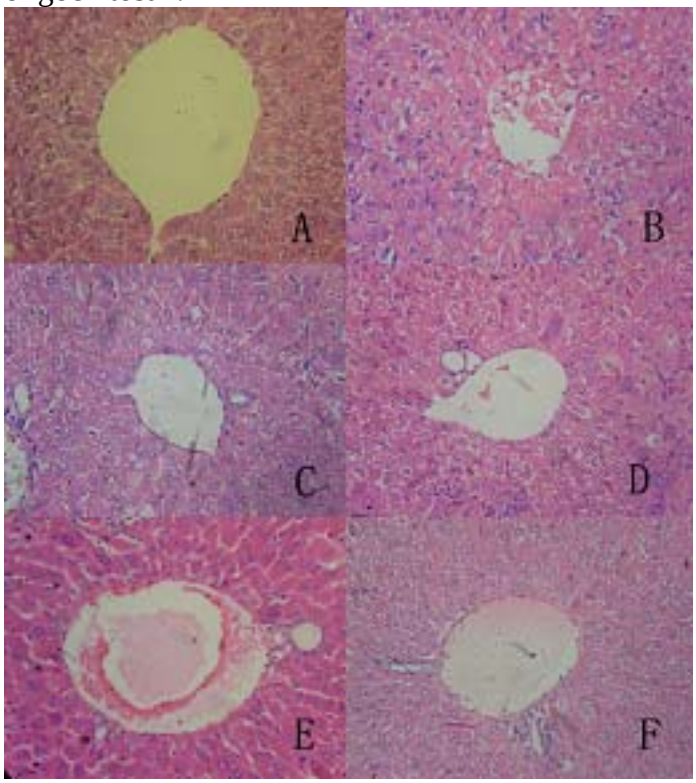

Figure 1 : Photomicrographs of liver sections taken from mice treated with CCl_4 with or without the pretreatment with COS. (A) Normal; (B) CCl_4;(C)COS+CCl_4 (50 mg/kg); (D)COS+CCl_4 (167 mg/kg); (E)COS+CCl_4 (500 mg/kg); (F) DDB+CCl_4 (200 mg/kg). (H&E stain, original magnification ×400)

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