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Protective effects of chitosan oligosaccharide and its derivatives against carbon tetrachloride-induced liver damage in mice

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Abstract

The protective effects of chitosan oligosaccharide (COS), D-glucosamine ($GlcNH_2$) and *N*-acetyl-D-glucosamine (GlcNAc) on carbon tetrachloride (CCl_4)-induced hepatotoxicity and the possible mechanisms that involved were investigated in male ICR mice. CCl_4 (20 mg/kg body weight, i.p.) administration induced marked increase in serum AST and ALT activities, primed liver lipid peroxidation, depleted sulfhydryl content, impaired total antioxidant capabilities and induced genotoxicity 24 h after administration. Pretreatment with COS, $GlcNH_2$, and GlcNAc (1.5 g/kg body weight, i.g.) for 12 consecutive days prior to CCl_4 challenge significantly induced metallothionein (MT) expression. Thus, the antioxidant defensive system in the body was strengthened to counteract the oxidative damage induced by the succedent CCl_4 administration. Serum AST and ALT activities were effectively decreased. Hepatic malondialdehyde formation was inhibited and sulfhydryl contents, total antioxidant capabilities were markedly restored. Genotoxicity as reflected by DNA fragmentation, however, was not mitigated by pretreatment with COS, $GlcNH_2$, and GlcNAc. Histophathologic results of liver also confirmed their hepato-protective effects. Pretreatment with COS, $GlcNH_2$, and GlcNAc also could significantly decrease serum creatinine and uric acid levels and inhibit lipid peroxidation in kidney homogenate. Our results suggest that pretreatment with COS, $GlcNH_2$, and GlcNAc can efficiently protect mice against CCl_4-induced toxicity.

Keywords: Carbon tetrachloride; Chitosan; Metallothionein; Lipid peroxidation

1. Introduction

Carbon tetrachloride (CCl_4)-induced liver damage has been extensively studied and widely used as a model for screening hepato-protectors. Because the damaging effects of CCl_4 are oxidative stress-involved, numerous investigations have been done about the hepato-protective effects through antioxidants [1–3]. Natural antioxidants are widely considered especially as preventive candidates for protection against chemical induced toxicity.

Chitosan is a cationic polysaccharide derived from *N*-deacetylation of chitin (Fig. 1), which is widely distributed in nature in the skeleton of crustaceans and is the second most abundant polymer after cellulose. Many hydroxyl and amino groups in the polysaccharide chain make chitosan easily modified. Many derivatives of chitosan with particular activities have been reported [4,5]. Chitosan is non-toxic and biodegradable, which make it an appealing biopolymer for researchers. The biological and pharmaceutical applications of chitosan have been reviewed [6–8]. Recently, the antioxidant activity of chitosan has received much attention. Xie et al. reported that chitosan could scavenge hydroxyl radicals [9]. Jeon showed that chitosan could decrease thiobarbituric acid reactive substances production and increase antioxidant enzymes activities in CCl_4 induced liver injury [10]. However, the antioxidant and hepato-protective activities of chitosan oligosaccharide (COS) and its derivatives including

Abbreviations: CCl_4, carbon tetrachloride; COS, chitosan oligosaccharide; $GlcNH_2$, D-glucosamine; GlcNAc, *N*-acetyl-D-glucosamine; T-AOC, total antioxidant capabilities; T-SH, total sulfhydryl; NP-SH, nonprotein sulfhydryl; MT, metallothionein; MDA, malondialdehyde

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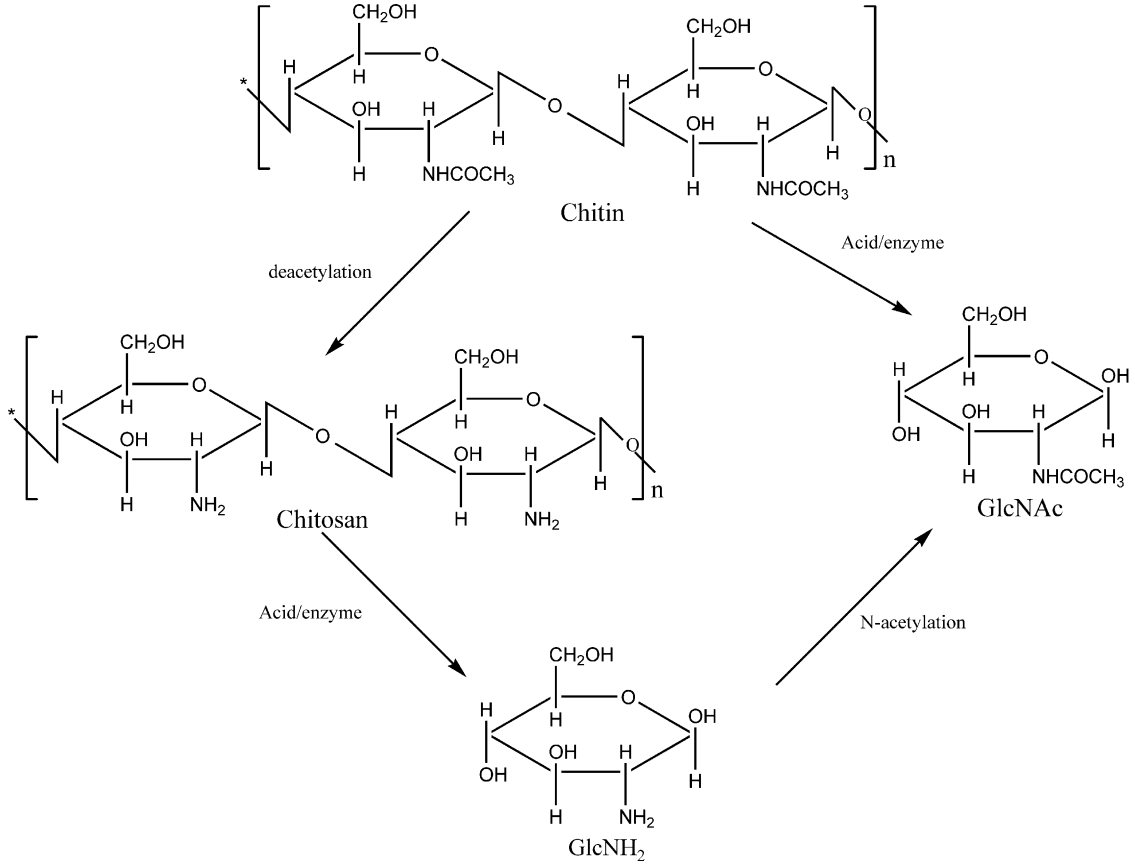

Fig. 1. Flow chart between chitin, chitosan, $GlcNH_2$ and GlcNAc.

D-glucosamine ($GlcNH_2$) and *N*-acetyl-D-glucosamine (GlcNAc) against CCl_4 induced liver damage and mechanisms that involved have not been addressed.

$GlcNH_2$ and GlcNAc are the monomer of chitosan and chitin, respectively, and they are attracting more researchers because of easy absorption. After oral administration, $GlcNH_2$ is completely ionized in the stomach and 98% of it is readily absorbed. The free $GlcNH_2$ that absorbed is then rapidly and selectively concentrated within organs particular liver (incorporation into plasma globulins) and kidney (urinary excretion), as well as cartilage. $GlcNH_2$ in organs will then exert favorable effects. $GlcNH_2$ was found to prevent the development of hepatocyte lysis syndrome and to normalize the cholate- and glycogen-synthetic functions of liver. $GlcNH_2$ exhibited significant anabolic effect and this may also contribute to its hepatoprotective activity [11].

The present study was undertaken to investigate the protective effects of COS, $GlcNH_2$, and GlcNAc against CCl_4 induced liver toxicity in mice by examining lipid peroxidation, total antioxidant capabilities (T-AOC), total sulfhydryl (T-SH), nonprotein sulfhydryl (NP-SH), and metallothionein (MT) contents in liver. Our results indicated that pretreatment with COS, $GlcNH_2$, and GlcNAc significantly enhanced body's antioxidant capability and thus prevent CCl_4-induced hepatotoxicity.

2. Chemicals and methods

2.1. Chemicals

CCl_4, olive oil, thiobarbituric acid, disodium EDTA, ethidium bromide, proteinase-K, DNAse-free RNAse-A, Tris, were obtained Qingdao Alp Science & Technology Co., Ltd. (Qingdao, China). DL2 000 DNA marker, 10× loading buffer and agarose were purchased from Takara. The total antioxidative capability (T-AOC) assay kit was purchased from Jiancheng Bioengineering Insititute (Nanjing, China). COS ($M = 3100$, purity > 98.6%), $GlcNH_2$ (purity > 99.5%) and GlcNAc (purity > 99.5%) were prepared in our lab.

2.2. Animals and treatment

Male ICR mice (25–30 g) from Qingdao Experimental Animal Center were used for this study. The animals were allowed free access to rodent chow and water and maintained in the natural environmental conditions without artificial light. The mice were acclimatized for at least 1 week prior to experiment. The experiment was done according to the Experimental Animal Committee Regulations.

Mice were randomly divided into eight groups with eight animals each. Group 1 was control group and animals received saline only for 12 days and olive oil (20 mg/kg,