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Effects of chitosan oligosaccharides on drug-metabolizing enzymes in rat liver and kidneys

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ABSTRACT

To investigate the effect of chitosan oligosaccharides (COS) on drug-metabolizing enzymes in rat liver and kidneys, male Spraque–Dawley rats were fed a diet containing 1% or 3% COS for 5 weeks. The activities of cytochrome P450 (CYP) enzymes, UDP-glucurosyltransferase (UGT) and glutathione *S*-transferase (GST) in the liver and kidneys were determined. Significant decreases in microsomal CYP3A-catalyzed testosterone 6β-hydroxylation, CYP2C-catalyzed diclofenac 4-hydroxylation, and CYP4A-catalyzed lauric acid 12-hydroxylation in the liver of rats fed the COS diets were observed compared with those rats fed the control diet. Immunoblot analyses of CYP proteins showed the same trend as with enzyme activities. Increased glutathione content in liver was found in rats fed the 1% COS diet. Increased hepatic NADPH: quinone oxidoreductase 1 (NQO1) activity was found in rats fed the COS diets. In kidneys, COS had little or no effect on CYP enzyme activities. However, increased GST activity was observed in rats fed the COS diets. Moreover, a higher UGT activity was found in rats fed the 1% COS diet. Our results indicate that COS may suppress hepatic CYP enzymes and induce phase II detoxifying reactions in the liver and kidneys of rats.

1. Introduction

Drug-metabolizing enzymes (DMEs) include phase I cytochrome P450 (CYP) oxidative and phase II conjugative enzyme systems. CYP monooxygenases are important phase I enzymes which catalyze different types of oxidative reactions and are responsible for the metabolism of various drugs and many endogenous substrates such as steroids, fatty acids and eicosanoids (Rendic, 2002). In general, drugs and chemicals undergo phase I oxidative metabolic reactions resulting in the formation of more water-soluble and less toxic metabolites. However, some CYP enzymes such as CYP 1A1, 3A, and 2E1 are involved in the metabolic activations of carcinogens such as benzo(a)pyrene, *N*-nitrosodimethylamine, and aflatoxin B1 (Gonzalez and Gelboin, 1994). Furthermore, CYP-mediated oxidative metabolism of the substrates may generate toxic electrophiles and reactive oxygen species (ROS) (Serron et al., 2000). Some pathophysiological alterations have been reported to modulate some CYP enzymes in liver. For instance, higher hepatic CYP2E1, CYP2B, CYP3A, and CYP4A expression was found in uncontrolled diabetes (Woodcroft and Novak, 1999). Steatosis produced by high fat diets rich in polyunsaturated fatty acids (Baumgardner et al., 2008) and that induced by chemicals such as ethanol or CCl_4 (Lieber, 1999) are established as the experimental models of diet and chemical-mediated liver injury, respectively. Those models have been reported to be associated with liver injuries via the alteration of hepatic expression of drug-metabolizing enzymes such as CYP2E1 and CYP4A.

Phase II enzymatic reactions, known as conjugation reactions, involve the addition of the intracellular polar groups including glucuronate, glutathione, sulfate, glycine to the foreign molecules (Rendic, 2002) and function to eliminate electrophiles and ROS generated by phase I reactions, thereby protecting organisms against chemical insult (Krajka-Kuźniak, 2007). Microsomal UDP-glucuronosyltransferase (UGT) and cytosolic glutathione *S*-transferase (GST) are two important phase II enzymes that catalyze the conjugation reactions resulting in the formation of water-

Abbreviations: COS, chitosan oligosaccharides; CYP, cytochrome P-450; DME, drug-metabolizing enzymes; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GSH, reduced glutathione; GST, glutathione *S*-transferase; GSSG, oxidized glutathione; HPLC, high-performance liquid chromatography; MS, mass spectrometric; NQO1, NAD(P)H quinone 1 oxidoreductase; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; TBARS, thiobarbituric acid reactive substances; UGT, UDP-glucuronosyltransferase.

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soluble glucuronate and glutathione conjugates to facilitate the excretion of xenobiotics. NADPH quinone 1 oxidoreductase (NQO1), a cytosolic flavoprotein catalyzing the two-electron reductive metabolism and detoxification of endogenous and exogenous chemicals (Ross, 2004), is critical for cytoprotection against many highly reactive and potentially damaging quinones, prevent redox cycling and protect cells against oxidative stress and neoplasia (Tsvetkov et al., 2005). Induction of phase II detoxifying enzymes and reduction of ROS was most pronounced in the prevention of chemical-induced tissue injuries and carcinogenesis (Kondraganti et al., 2008). Activity and gene transcription of CYP isozymes and phase II enzymes have been demonstrated to be susceptible for modulating by numerous chemicals including environmental pollutants, heavy metals, organic solvents, and also by dietary components, such as fatty acids, α-tocopherol, sulfur-containing compounds, and dietary fibers (Chen et al., 2003; Traber et al., 2005; Yao et al., 2006; Nugon-Baudon et al., 1996; Sugatani et al., 2006; Yao et al., 2010). Induction or inhibition of DMEs may change the pharmacological activities and toxicities of drugs and carcinogens and may also cause drug interactions (Guengerich, 1997).

Chitosan is a polysaccharide that is known to have important functional properties in many areas but its applications as functional foods are restricted due to poor absorption. Unlike chitosan, chitosan oligosaccharides (COS), the hydrolyzed product of chitosan, is a mixture of oligomers of β-1,4-linked D-glucosamine residues that is readily soluble in water due to their shorter chain lengths and free amino groups in D-glucosamine units (Jeon and Kim, 2000). Numerous pharmacological activities of COS have been demonstrated in cell-based assays but relatively less animal or human studies have been performed. Recent advances indicate that COS has many desirable biological activities including anti-diabetes activity (Kumar et al., 2009), antioxidant activity (Chen et al., 2003), free radical scavenging activity (Mendis et al., 2007), anti-inflammation (Yoon et al., 2008), immune modulation activity (Ding et al., 2010) and antimutagenic activity (Shen et al., 2009). In addition, COS has also been shown to increase phase II detoxification enzymes such as NQO1 and GST (Nam et al., 2007; Shon and Nam, 2005), and induce intracellular GSH level (Mendis et al., 2007; Nam et al., 2007; Senevirathne et al., 2011) in various cell lines. These results suggest that COS may act as a chemopreventive agent against carcinogenesis and oxidative stress-related pathologies.

Most drug metabolism occurs in the liver, but other organs such as kidneys also may play a role. To date, it is still unknown whether COS could modulate DME activity in both liver and kidneys, resulting in changes of the pharmacological activities and toxicities of chemicals. In this study, rats were fed with COS and changes on CYP and phase II detoxification enzymes, GST, UGT and NQO1 in the liver and kidneys were investigated.

2. Materials and methods

2.1. Materials

Testosterone, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, resorufin, *p*-nitrophenol, 4-nitrocatechol, NADPH, glutathione, 1-chloro-2,4-dinitrobenzene, sodium dodecylsulfate (SDS), cytochrome *c*, heparin, Ponceau S and cellulose were obtained from Sigma (St. Louis, MO, USA). 6-beta-Hydroxytestosterone was purchased from Ultrafine Chemicals (Manchester, UK). Atorvastatin was purchased from Waterstone Technology, LLC (Carmel, IN, USA). 2-OH- and 4-OH atorvastatin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other chemicals and reagents were of analytical grade and were obtained commercially. Chitosan oligosaccharides (COS), prepared from shrimp shell chitosan (95% deacetylation), was generously supplied by the Taiwan Tanabe Seiyaku Company (Taipei, Taiwan). COS standards, a mixture of oligomers, including glucosamine and chitosan dimer, trimer, tetramer, pentamer and hexamer were purchased from Seikagaku Corp. (Tokyo, Japan). The composition of the COS was analyzed by HPLC methods (Hicks, 1988; Izume and Ohtakara, 1987) using a refractive index (RI) detector and an Inertsil NH_2 column (5 μm, 4.6 × 250 mm). The flow rate was 1 mL/min and the mobile phase was acetonitrile–water (60:40). Identification of the COS was carried out by comparing the retention time of different oligomers with that of the standards under the same HPLC conditions (Fig. 1). Each gram of COS contains: glucosamine, 4.0 mg; chitosan dimer, 66.5 mg; chitosan trimer, 137.1 mg; chitosan tetramer, 148.2 mg; pentamer, 178.8 mg and hexamer, 53.1 mg; others: 412.3 mg.

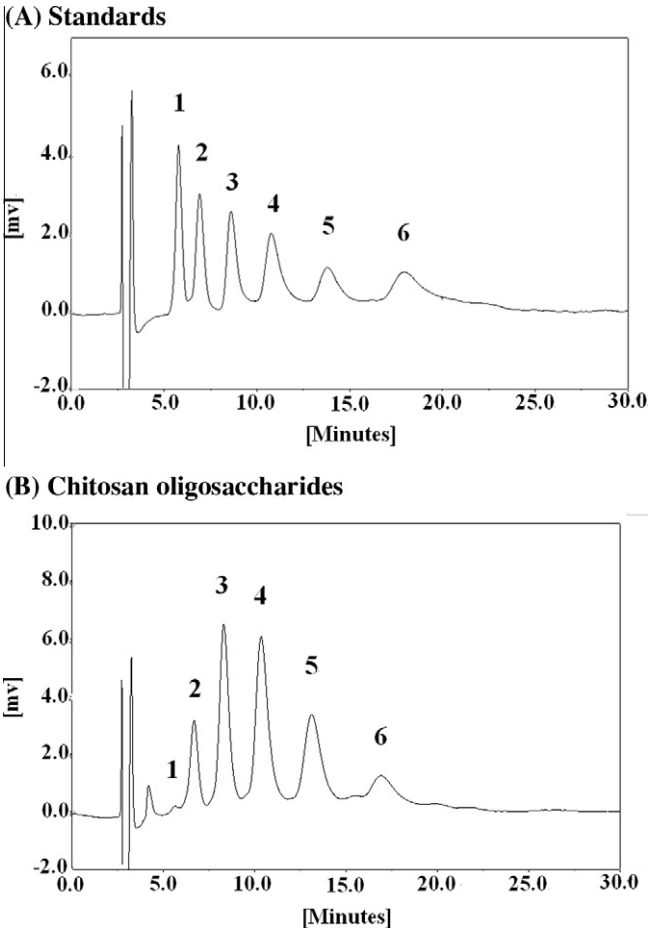

Fig. 1. HPLC chromatography of chitosan oligosaccharides (COS). (A) COS standards (0.33 mg/mL), (B) COS sample (5.5 mg/mL). Retention times: glucosamine (Peak 1), 5.8 min; chitosan dimer (Peak 2), 6.9 min; chitosan trimer (Peak 3), 8.6 min; chitosan tetramer (Peak 4), 10.7 min; chitosan pentamer (Peak 5), 13.6 min; chitosan hexamer (Peak 6), 17.6 min.

2.2. Animal study

Male Spraque–Dawley rats weighing 250 g (6 weeks old) were obtained from BioLASCO, Taiwan (Ilan, Taiwan). During the adaptation period, rats were fed a chow diet for 1 week. Then the animals were randomly divided into control, 1% and 3% COS groups with eight rats in each group. The compositions of the experimental diets given to the control test animals are 20% casein, 10% soybean oil, 1% vitamin mixture, 4% mineral mixture, 0.2% choline chloride, 4% cellulose and 60.8% corn starch. The vitamin and mineral mixtures (AIN 93) were purchased from ICN Biochemicals (Costa Mesa, CA). The other two group animals were fed the same diet containing 1% or 3% COS. Rats were housed in plastic cages in a room kept at 23 ± 1 °C and 60 ± 5% relative humidity with a 12-h light and dark cycle. Food and drinking water were available *ad libitum* for 5 weeks. This study was approved by the Animal Center Management Committee of China Medical University. The animals were maintained in accordance with the guidelines for the care and use of laboratory animals as issued by the Animal Center of the National Science Council, Taiwan.

2.3. Collection of blood and tissue samples

At the end of the experimental period, animals were fasted for 12 h prior to being sacrificed. Animals were killed by exsanguination via the abdominal aorta while under carbon dioxide (70%/30%, CO_2/O_2) anesthesia. Heparin was used as