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Dose- and time-dependent effects of luteolin on carbon tetrachloride-induced hepatotoxicity in mice

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Abstract

Carbon tetrachloride (CCl_4) is a well-known model compound for producing chemical hepatic injury. This study investigated the protective effects of the flavonoid luteolin on the CCl_4-induced hepatotoxicity in mice. Luteolin dissolved in dimethyl sulfoxide (DMSO) was administered intraperitoneally (i.p.) at 5 or 50 mg/kg as a single dose, and once daily for 2 consecutive days. Two hours after the final treatment, the mice were treated with CCl_4 (20 mg/kg, i.p.). CCl_4-induced hepatotoxicity was reduced in a dose- and time-dependent manner, as determined by decreased serum aminotransferase activities and liver histopathology. CCl_4 intoxication resulted in an overexpression of heat shock protein gp96 in the mice liver, which was strongly attenuated by luteolin pretreatment. Luteolin has also decreased oxidative stress produced by CCl_4, as suggested by improvement in the Cu/Zn superoxide dismutase activity. The effect of luteolin on myeloperoxidase, an indicator of inflammatory cell infiltration, was also investigated. Treatment of the mice with luteolin resulted in a significant decrease in the myeloperoxidase activity. The hepatoprotective effect of luteolin against CCl_4 hepatotoxicity was higher in animals pretreated with luteolin for 2 consecutive days. This suggests that the protection might be due to induction of some adaptive mechanisms. The data indicate that luteolin could be effective in protecting mice from the hepatotoxicity produced by CCl_4.

Keywords: Carbon tetrachloride hepatotoxicity; Luteolin; Cu/Zn superoxide dismutase; Myeloperoxidase; Histopathology; Time- and dose-dependent; Heat shock protein; gp96

Introduction

The liver plays a central role in transforming and clearing metabolites and xenobiotics, and is susceptible to the toxicity from these agents. Carbon tetrachloride (CCl_4) is a well-known model compound for inducing liver injury. Its biotransformation by the hepatic microsomal cytochrome P450 produces hepatotoxic metabolites, namely trichloromethyl free radicals. The covalent binding of the trichloromethyl free radicals to the cell proteins is considered to be the initial step in a chain of events that eventually lead to cell necrosis (Recknagel et al., 1989; Williams and Burk, 1990; Brautbar and Williams, 2002). During liver damage, inflammatory cell infiltration may occur, which can be quantified by measuring the activity of myeloperoxidase (MPO), an enzyme found within the azurophilic granules of polymorphonuclears such as neutrophils and monocyte/macrophage mononuclears, including Kupffer cells (Reynolds et al., 2002).

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Flavonoids, a large class of polyphenolic compounds present in fruits and fruit products, vegetables, and plant-derived beverages such as tea and wine, are associated with a variety of beneficial properties (Middleton et al., 2000; Havsteen, 2002). Due to their antioxidant activity (Rice-Evans et al., 1996), they are presumed to protect tissues against oxidative stress and associated pathologies such as cancer, coronary artery disease and inflammation. Polyphenols act as antioxidants by scavenging reactive oxygen and nitrogen species, chelating redox-active transition metal ions preventing them from catalyzing free radical formation, but also several other ways (Frei and Higdon, 2003). Luteolin (3′,4′,5,7-tetrahydroxyflavone) is an important member of the flavonoid family, present in glycosylated forms and as aglycone in various plants. Luteolin is reported to have antiinflammatory (Ziyan et al., 2007; Ueda et al., 2002), antioxidant (Perez-Garcia et al., 2000), antiallergic (Veda et al., 2002), antitumorigenic (Ju et al., 2007), anxiolytic-like (Coleta et al., 2007), and vasorelaxative properties (Woodman and Chan, 2004).

Heat shock proteins (HSPs) are induced in response to various stresses and to protect cells from such stresses (Lee et al., 2004). The 90 kDa HSP family, one of the most abundant proteins in eukaryotic cells, plays an important role in the folding of newly synthesized proteins and stabilization and refolding of denatured proteins in stress conditions (Sreedhar et al., 2004). The progressive cellular damage caused by reactive oxygen species contributes to protein misfolding and accumulation of HSPs. HSP gp96 (Grp94, glucose regulated protein 94) is a constitutively expressed endoplasmic reticulum lumenal protein that is upregulated in response to cellular stress such as heat shock, oxidative stress or glucose depletion (Ruddon and Bedows, 1997). HSP gp96 has not been previously studied in toxic liver damage.

The main purpose of this study was to investigate the potential effects of luteolin in reducing oxidative stress and inflammation in the liver of mice caused by CCl_4, as well as enhancement of hepatic proliferative capability, which could provide helpful information for the prevention of liver damage. The present investigation focuses on evaluation of the efficacy of different dose regimes of luteolin for their protection against CCl_4-induced hepatotoxicity. The parameters analysed were serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, histopathology, HSP gp96 protein expression, hepatic Cu/Zn superoxide dismutase (Cu/Zn SOD) and myeloperoxidase activities.

Materials and methods

Olive oil was provided by Zvijezda, Croatia. Luteolin, carbon tetrachloride, diagnostic kits for the serum alanine aminotransferase and aspartate aminotransferase, dimethyl sulfoxide (DMSO), hexadecyltrimethylammonium bromide (HTMABr), *o*-dianisidine dihydrochloride, hydrogen peroxide (H_2O_2) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Superoxide Dismutase Assay Kit was obtained from Cayman Chemical (Ann Arbor, MI, USA). All other chemicals and solvents were of the highest grade commercially available.

Animals

Male Balb/c mice from our breeding colony, 2–3 month old, were divided into 8 groups with 6 animals per group. Mice were fed a standard rodent diet (pellet, type 4RF21 GLP, Mucedola, Italy), and water *ad libitum*. The animals were maintained at 12 h light/dark cycle, at constant temperature (20 ± 1 °C) and humidity (50 ± 5%). All experimental procedures were approved by the Ethical Committee of the Medical Faculty, University of Rijeka.

Experimental design

The control group animals were given DMSO as a single dose (group I) or once daily for 2 consecutive days (group II). Luteolin dissolved in DMSO was administered intraperitoneally (i.p.) at 5 or 50 mg/kg as a single dose (groups III and IV), and once daily for 2 consecutive days (groups V and VI). Groups VII and VIII received vehicle (DMSO) as a single dose and once daily for 2 consecutive days, respectively. Two hours after the final treatment, the mice were treated with CCl_4 (20 mg/kg, i.p., dissolved in olive oil), except the control groups.

Twenty-four hours after administrating CCl_4, the mice were killed by cervical dislocation, blood was collected from the orbital sinus and the serum was separated to determine ALT and AST activities. The abdomen was cut open quickly and the liver was perfused thoroughly with isotonic saline, excised, blotted dry and divided into multiple samples. Liver samples were used to assess the Cu/Zn SOD and MPO activities, and protein content. A portion of the livers were preserved in a buffered formalin solution to obtain the histological sections.

Hepatotoxicity studies

Serum levels of ALT and AST, as markers of hepatic function, were measured by using a Bio-Tek EL808 Ultra Microplate Reader (BioTek Instruments, Winooski, VT, USA) according to manufacturer's instructions.