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The hepatoprotective effect of lycopene on Con A-induced liver injury in mice

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Lycopene, the main fat-soluble pigment responsible for the red color of ripe tomatoes, is a symmetrical tetraterpene comprising eight isoprene units. *In vitro* and *in vivo* studies have shown that lycopene acts as a potent antioxidant; it is 100 times more effective than vitamin E and 125 times more effective than glutathione as an antioxidant. Here, we divided BALB/c male mice into three equal groups: control, Concanavalin A (Con A), and Con A and lycopene. The control group mice received only vehicle by intraperitoneal injection, the Con A group mice were given Con A, and the Con A and lycopene group mice received Con A and lycopene. The results showed that Con A administration increased histopathological damage, and the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), interleukin (IL)-6, interferon (IFN)-γ and tumor necrosis factor (TNF)-α were increased in serum samples whereas the levels of these compounds were significantly decreased in the Con A and lycopene group compared to the Con A group. Furthermore, we observed that lycopene led to an increase in cell viability and cell growth. The results of this study revealed that lycopene might be a useful hepatoprotective agent for reducing increased proinflammatory cytokine levels, and for increasing cell viability and cell growth.

1. Introduction

Many physiologically active hepatoprotective compounds, such as those with antifibrotic activity, have been found in tea, fruits, and vegetables (Clinton 1998; Mendel 2013). Lycopene is the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and of tomato products. Lycopene can quench active oxygen 100 times more efficiently than vitamin E and 125 times more efficiently than glutathione (GSH) (Mein et al. 2008), and is the most efficient quencher of active oxygen of all naturally occurring carotenoids identified to date (Kumar et al. 2009). Consequently, lycopene has recently been in great demand as a food additive and a natural antioxidant. Additionally, lycopene also exhibits potent neuroprotective, anti-inflammatory, and anti-proliferative activities, helps maintain normal cell metabolism, and decreases the risk of chronic diseases such as cardiovascular diseases and cancer (Hansson et al. 2006; Bae et al. 2011; Haddad et al. 2013; Qiu et al. 2013; Mikako et al. 2014; Asokan et al. 2015). Carcinogenesis from chronic hepatitis is thought to occur because of persistent inflammation of the liver caused by viral hepatitis (hepatitis B virus and hepatitis C virus) and the associated fibrosis, necrosis, and generation of tissue. The inhibition of liver inflammation was examined to determine whether anti-inflammation agents can prevent hepato-carcinogenesis. Glycyrrhizin is a widely used anti-inflammatory agent isolated from licorice root that inhibits liver inflammation and decreases hepatic enzyme levels (Nishimoto et al. 2010). However, there are few drugs that can be used for hepatoprotective therapy. In addition, the onset of liver cancer due to nonalcoholic steatohepatitis (NASH) has recently become problematic. Thus, there is a need for new therapeutic strategies to address liver inflammation (Itoh et al. 2009, 2010). The intravenous injection of concanavalin A (Con A) into mice increases the level of plasma alanine aminotransferase (ALT); simultaneously, activated T cells infiltrate the liver, followed by the apoptosis and necrosis of hepatocytes. The activation of T cells by Con A results in increased levels of inflammatory cytokines, including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-6. In the present study, we examined the liver protective effect of lycopene using a hepatitis mouse model. We found that lycopene could suppress Con A-induced liver inflammation and damage in mice.

2. Investigations, results and discussion

We first investigated the hepatoprotective effect of lycopene on Con A-induced liver injury in mice. Lycopene (25 mg/kg) was injected intraperitoneally just before Con A injection. We measured the hepatic transaminase levels and pro-inflammatory cytokines in serum after injection to determine the hepatotoxicity due to Con A administration. Twenty-four hours after Con A treatment, the titers of serum ALT and AST were greatly increased as compared to the untreated control (Fig. 1A). The intraperitoneal administration of lycopene significantly decreased the serum ALT and AST levels. A previous study reported that lycopene could reduce ALT and AST activity against carbon tetrachloride-induced acute liver injury in rat (Kim et al. 2004). Oxidative stress induced by carbon tetrachloride causes cytotoxicity, which leads to apoptosis or necrosis in liver tissue (Suryanarayana et al. 2011). We obtained histological evidence for protection from liver injury by lycopene by preparing liver sections and staining with hematoxylin and eosin; representative images are shown in Fig. 1B to 1D. Mice administered Con A suffered from severe liver damage, as indicated by inflammatory infiltration around the central veins (Fig. 1B), but this infiltration was completely blocked in mice pretreated with lycopene. These findings suggest that lycopene might be effective in the treatment of Con A-induced hepatotoxicity.

We next evaluated the serum levels of several proinflammatory cytokines and measured the serum IL-6, IFN-γ and TNF-α levels 3 and 6 h after Con A treatment (Fig. 2). All three levels were significantly increased in the Con A group compared with the control group. Cytokines are polypeptide molecules secreted to protect against micro-organisms and other antigens to regulate immune and inflammatory responses. IFN-γ plays important roles in the inflammatory processes and is an important activator of macrophages (Abbas et al. 1999). TNF-α plays an active role

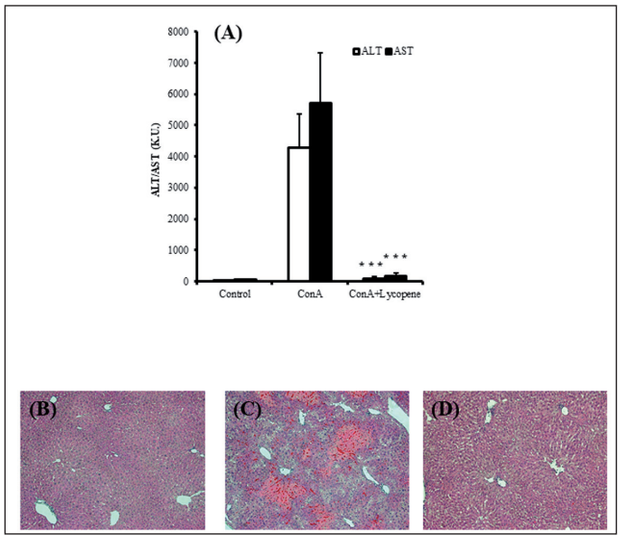

Fig. 1: Protective effect of lycopene on Con A-induced liver injury. Mice were administrated an intravenous injection of Con A (30 mg/kg) and an intraperitoneal injection of lycopene (25 mg/kg). Sections of paraffin-embedded liver tissue were stained with hematoxylin and eosin. The liver was excised from control (A), Con A-treated (B), and Con A and lycopene-treated (C) mice. Whole blood was collected to determine the serum levels of ALT (open columns) and AST (solid columns) (D). The values are the mean ± SEM (n=4). * * *$p < 0.001$ as compared to Con A treatment alone.

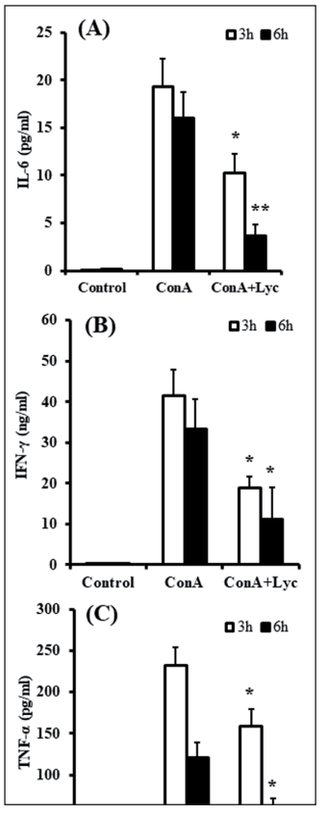

Fig 2: Changes in serum IL-6, IFN-γ and TNF-α levels measured by ELISA. Mice received an intravenous injection of Con A and an intraperitoneal injection of lycopene. Cytokine levels were measured 3 and 6 h after Con A treatment. Serum IL-6 (A), IFN-γ (B), and TNF-α (C) levels in the different experimental groups. Open and solid columns (D) indicate the Con A treatment alone and lycopene treatment groups, respectively. The values are the mean ± SEM (n=4). *$p < 0.05$, **$p < 0.01$ as compared to Con A treatment alone.

in inflammation, is produced by activated macrophages, and is involved in systemic inflammation and mediates acute inflammation. Administration of Con A has been shown to increase TNF-α levels (Ksontini et al. 1998). The biological role of IL-6 is not clear, but it appears to act as a proinflammatory or anti-inflammatory cytokine. There is strong evidence of the importance of IL-6 in the modulation of inflammation in different experimental models, e.g., balancing the levels of proinflammatory cytokines. The intraperitoneal injection of 25 mg/kg of lycopene significantly decreased the levels of IL-6, IFN-γ and TNF-α in serum, clearly indicating that lycopene has a protective effect against Con A-induced liver injury. We also analyzed the number of hepatocytes on day 2 and day 3 for the control and treatment groups and found that the cell numbers showed significant differences in the two groups (Fig. 3A to 3E).

We have shown that lycopene can decrease Con A-induced hepatic injury *in vivo*. These effects suggest that lycopene has antioxidant and cell protective effects. Liver toxicity induced by Con A is caused by oxidative stress. Mein et al (2008) reported that lycopene has antioxidant effects in the liver. Antioxidants can reduce the damaging effects caused by oxidative stress on DNA, proteins, lipids and the cell membrane. Therefore, ascorbic acid, tocopherol and N-acetylcysteine have been used as antioxidants to overcome the hepatotoxicity caused by Con A, and beneficial effects have been identified. These effects were achieved by reducing oxidative stress. We confirmed that lycopene has hepatocyte-protective effects (Fig. 3E).

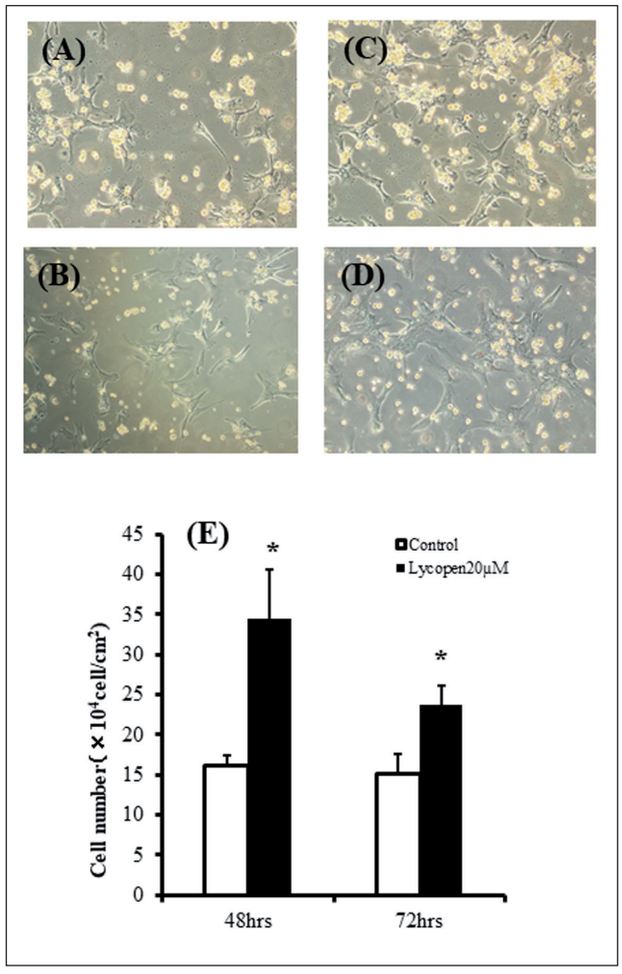

Fig. 3: Lycopene increases hepatocyte growth of cultured hepatocytes. Freshly isolated hepatocytes treated with 20 μM lycopene for 48 or 72 and microscopy phase images of the cells were obtained under control conditions (A and C) or of cells treated with lycopene (B and D). The cell number was counted after collection (E). The values are the mean ±SEM (n=4). *$p < 0.05$ versus control.

This report is the first to indicate the hepatoprotective effects of lycopene towards damage induced by Con A. Further evaluation of the hepatoprotective effects of lycopene is clearly required prior to its clinical application as a liver-protective therapy.

3. Experimental

3.1. Materials

Lycopene, corn oil and dimethyl sulfoxide (DMSO) were obtained from WAKO Pure Chemical Industries (Osaka, Japan). Concanavalin A (Con A) from jack beans was purchased from Calbiochem (San Diego, CA, USA).

3.2. Animals

Eight-week-old BALB/c male mice and 6-week old Sprague Dawley (SD) male rats were purchased from Funabashi Farm Co., Ltd. (Chiba, Japan). The animals were maintained in a controlled environment (temperature: 23±1.5 °C; light: 12-h light/dark cycle) with free access to standard rodent chow and water. The mice and rats were given 1 week to adapt before commencing the experiments. The experimental protocols conformed to the ethical guidelines of the Faculty of Pharmaceutical Sciences, Tokyo Heisei University, Japan.

3.3. Isolation of hepatocytes from rat liver

Hepatocytes were isolated from SD rats by the collagen perfusion method described by Seglen (1976). The cells were suspended in Williams' E medium containing 10% fetal calf serum, 1 nM insulin, and 1 nM dexamethasone. Cell viability was assessed by Trypan blue dye exclusion. Cells that were at least 85% viable were used in this study. The suspended cells were seeded onto a 6-well plate at 1.0×10^6 cells/well for the lycopene protection assay and cultured in a humidified 5% CO_2 incubator at 37 °C.

3.4. Biochemical analysis

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) levels were measured using commercially available kits according to the manufacture's protocol (Wako Pure Chemical Industries). The levels of IL-6, TNF-α and IFN-γ in serum samples were determined using a mouse enzyme-linked immunosorbent assay (ELISA) kit (Biosource, San Jose, CA, USA) according to the manufacturer's instructions. Blood samples were collected at 3 and 6 h because the cytokine levels increased more rapidly than the transaminase levels and essentially returned to normal levels within 12 h.

3.5. Histological analysis

The liver was removed and fixed with 4% paraformaldehyde. After sectioning, thin sections of tissues were stained with hematoxylin and eosin for histological observation.

3.6. Statistical analysis

Statistical analyses were performed with Microsoft Excel with the Statcel add-in (EMS Publication Co., Ltd., Saitama, Japan). All data are presented as means ± SEMs. The significance of differences between the control and experimental groups was assessed using the Dunnett test. A P value <0.05 was considered indicative of statistical significance.

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Conflicts of interest: None declared.

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Conflicts of interest: The authors declare that they have no competing interests.