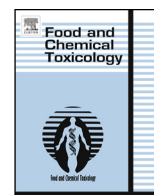
ARTICLE IN PRESS

Food and Chemical Toxicology ■■ (2014) ■■–■■

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox



# Differential protective effects of extra virgin olive oil and corn oil in liver injury: A proteomic study

Hualin Wang [a,b], Wat-Hung Sit [b], George Lim Tipoe [c], Jennifer Man-Fan Wan [b,*]

[a] School of Biology and Pharmaceutical Engineering, Wuhan Polytechnic University, Wuhan, Hubei, China
[b] Food and Nutrition Division, School of Biological Sciences, The University of Hong Kong, Hong Kong, China
[c] Department of Anatomy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

ARTICLE INFO

*Article history:*
Received 14 November 2013
Accepted 2 September 2014
Available online

*Keywords:*
Extra virgin olive oil
Corn oil
Carbon tetrachloride
Liver
Proteomics

ABSTRACT

Extra virgin olive oil (EVOO) presents benefits against chronic liver injury induced by hepatotoxins such as carbon tetrachloride ($CCl_4$); however, the protective mechanisms remain unclear. In the present study, a two-dimensional gel based proteomic approach was constructed to explore the mechanisms. Rats are injected with $CCl_4$ twice a week for 4 weeks to induce liver fibrosis, and were fed laboratory chow plus 20% (w/w) of either corn oil or EVOO over the entire experimental period. Histological staining, MDA assay and fibrogenesis marker gene analysis illustrate that the $CCl_4$-treated animals fed EVOO have a lower fibrosis and lipid peroxidation level in the liver than the corn oil fed group. The proteomic study indicates that the protein expression of thioredoxin domain-containing protein 12, peroxiredoxin-1, thiosulphate sulphurtransferase, calcium-binding protein 1, Annexin A2 and heat shock cognate 71 kDa protein are higher in livers from EVOO-fed rats with the $CCl_4$ treatment compared with those from rats fed with corn oil, whereas the expression of COQ9, cAMP-dependent protein kinase type I-alpha regulatory subunit, phenylalanine hydroxylase and glycerate kinase are lower. Our findings confirmed the benefits of EVOO against chronic liver injury, which may be attributable to the antioxidant effects, hepatocellular function regulation and hepatic metabolism modification effects of EVOO.

© 2014 Elsevier Ltd. All rights reserved.

## 1. Introduction

The liver plays a central role in the body by detoxifying enthetic chemical molecules through oxidation, reduction and/or conjugation (Grant, 1991). Meanwhile, long-term exposure to toxic substances, such as alcohol or certain drugs, may induce liver injury (Stickel et al., 2002). Hepatitis viral infection, notably with hepatitis B virus (HBV) and hepatitis C virus (HCV), is another major cause of chronic liver injury (Fung et al., 2007; Yao et al., 2005). Chronic liver diseases, including chronic HBV and/or HCV infection, alcoholic liver disease, non-alcoholic fatty liver disease and primary biliary cirrhosis, are major threats to human health in mainland China and Hong Kong (Fan and Farrell, 2009; Fung et al., 2007; Yao et al., 2005). Most types of chronic liver diseases will lead to liver inflammation, hepatocellular dysfunction, activation of hepatic stellate cells (HSCs) and accumulation of extracellular matrix, which is known as liver fibrosis, then further develop to cirrhosis and even hepatocellular carcinoma (Bataller and Brenner, 2005; Parsons et al., 2007). The liver has regenerative potential, and hepatic fibrosis is reversible under appropriate treatment (Bataller and Brenner, 2005). Animal liver fibrosis models assist the study of the effects of medicine and/or nutrients against chronic liver injury; carbon tetrachloride ($CCl_4$) induced liver fibrosis is the most widely used model in rodents (Constandinou et al., 2005). The detoxification of $CCl_4$ occurs in livers by cytochrome P-450. This process releases abundant peroxy free radicals, resulting in lipid peroxidation and liver injury. Chronic intake of $CCl_4$ will lead to liver fibrosis and cirrhosis (Constandinou et al., 2005; Slater et al., 1985). In the present study, the $CCl_4$ induced bridging liver fibrosis rat model was selected to study the effects of two dietary oils, extra virgin olive oil (EVOO) and corn oil, on chronic liver injury.

The liver is a major organ in which fatty acid metabolism occurs (Frayn et al., 2006). Ingested dietary lipids are digested and absorbed in the intestine, then delivered into the liver via the portal vein and metabolized in hepatocytes (Bradbury, 2006). Olive oil, a widely applied omega-9 enriched dietary lipid, has attracted much interest in its effects against liver injuries. Several *in vivo* studies have illustrated the hepatoprotective effects of EVOO against $CCl_4$ induced liver injury (Fang et al., 2008; Tanaka et al., 2009). A typical EVOO contains 80% of omega-9 monounsaturated fatty acid (MUFA), oleic acid, and other minor components such as aliphatic alcohols, sterols and polyphenols (e.g., α-tocopherols and hydroxytyrosol) (Huang and Sumpio, 2008). Oleic acid reduces the expression of α-SMA and presents an anti-apoptosis effect in hepatocytes (Ricchi

* Corresponding author. Food and Nutrition Division, School of Biological Science, The University of Hong Kong, Hong Kong, China. Tel.: +852 2299 0832.
*E-mail address:* jmfwan@hku.hk (J.M.-F. Wan).

http://dx.doi.org/10.1016/j.fct.2014.09.002
0278-6915/© 2014 Elsevier Ltd. All rights reserved.

Please cite this article in press as: Hualin Wang, Wat-Hung Sit, George Lim Tipoe, Jennifer Man-Fan Wan, Differential protective effects of extra virgin olive oil and corn oil in liver injury: A proteomic study, Food and Chemical Toxicology (2014), doi: 10.1016/j.fct.2014.09.002

et al., 2009; Tanaka et al., 2009). Meanwhile, several studies focus on the antioxidant effects of the phenol components in EVOO; these studies demonstrate the hepatoprotective effects against oxidative stress *in vitro* and *in vivo* (Deiana et al., 2007; Goya et al., 2007; Sarria et al., 2012). Additionally, the phenol components have the potential to relieve liver fibrosis (Vazquez-Martin et al., 2012). However, corn oil, one of the most widely utilized dietary lipid that is rich in omega-6 polyunsaturated fatty acids (PUFAs), has much fewer benefits against liver damage, and may enhance the inflammatory response and lipid peroxidation, even lead to aggressive liver injury (Fang and Lin, 2008; Hwang, 2009).

However, most of these studies investigate the histopathological alteration, antioxidant effects and fibrogenic related gene expression change. The present study aims to provide a deep insight into the differential effects of high EVOO or corn oil consumption on the hepatic proteome alteration in $CCl_4$-induced liver injury. These insights are gained from a two-dimensional electrophoresis (2-DE)-based proteomic study. Proteomics is a powerful tool to investigate the protein expression change and to assist the exploration of the potential molecular mechanisms (de Roos and Romagnolo, 2012; Duncan and Hunsucker, 2005). Our findings can assist the understanding of the mechanisms by which EVOO protects the liver against hepatic fibrogenesis.

## 2. Materials and Methods

### 2.1. Animal experiments

Five-month-old male Fischer 344 rats (weighing approximately 450 g) were obtained from the Animal Resources Centre, Western Australia and bred in the Animal House, School of Biological Sciences, The University of Hong Kong. All rats were kept individually in stainless steel cages in an animal room. The rats experienced a 12-h/12-h light/dark cycle with controlled temperatures (22 °C) and humidity (50%). Animals were provided water *ad libitum*. After 1 week of acclimatization, the rats were arbitrarily divided into four groups: corn oil normal group (corn oil $CCl_4$–), olive oil normal group (olive oil $CCl_4$–), corn oil $CCl_4$ group (corn oil $CCl_4$+) and olive oil $CCl_4$ group (olive oil $CCl_4$+). The rats in the two corn oil groups were fed high corn oil diets (20% corn oil mixed with normal rodent chow 5053, LabDiet, USA) *ad libitum*, and the rats in the olive oil groups were fed high olive oil diets (20% extra virgin olive oil mixed with rodent chow 5053). All rats were fed high lipid diets for 1 week, and then the rats in the two $CCl_4$+ groups received $CCl_4$ (0.1 mL/100 g body weight, 1:1 mixed with soybean oil) by subcutaneous administration twice a week for 4 weeks; the rats in the $CCl_4$– groups were given soybean oil only. The high lipid diets were continuously given over the entire experimental period. Twenty-four hours after the last injection, all rats were killed. The liver tissue were excised and weighed. The right lob were harvested, fixed in 10% buffered formalin solution (Surgipath, Germany) for histological staining or snap frozen in liquefied nitrogen and stored at –80 °C for further investigations.

### 2.2. Histological staining

The fixed liver tissues were embedded in paraffin and sectioned at 5-μm thicknesses. Sections were stained with Masson's trichrome for collagen (Sigma-Aldrich, USA). The stained slides were observed photomicrographically, and the degree of liver damage was examined blindly under a Nikon N80i microscope (Nikon, Japan), evaluated with the Knodell histological activity index (Knodell et al., 1981) modified as follows: 0, no fibrosis; 1, portal fibrosis; 2, fibrous portal expansion; 3, bridging fibrosis (portal–portal or portal–central linkage); and 4, cirrhosis.

### 2.3. Malondialdehyde (MDA) examination

The lipid peroxidation was quantitatively measured by estimating the concentration of thiobarbituric acid reactive substances (TBARS) in the liver. The quantity of MDA formed was measured by the reaction with thiobarbituric acid (TBA) (Buege and Aust, 1978). Liver samples were homogenized in ice and centrifuged to remove cell debris. The supernatant were mixed with a TBA reagent consisting of 0.8% TBA in 10% acetic acid (Merck, Germany). The reaction mixtures were incubated in a boiling water bath for 45 min, cooled and centrifuged for 5 min. Supernatants were collected and the absorbance of the coloured layer was measured by a spectrophotometer at 532 nm. The results were expressed as MDA nmol/mg protein.

### 2.4. Reverse transcription PCR

Reverse transcription (RT)-PCR was employed to analyse the differential gene expression at a transcriptional level. Total RNA was extracted from the liver samples of the four groups using TRIzol (Invitrogen, USA). Aliquots of the total RNA (5 μg) were reverse-transcribed with Super Script III (Invitrogen) according to the manufacturer's instructions. PCR was performed with an iCycler Thermal Cycler (Bio-Rad, USA). cDNA (0.5 μL) were used for each PCR amplification in a total reaction volume of 15 μL, and all reactions were performed in duplicate. The ubiquitin C transcript was adopted as an internal control. The amplification was initiated by 4 min of denaturation at 94 °C for one cycle, followed by 30 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min. After the last cycle of amplification, samples were incubated for 7 min at 72 °C. The PCR products were examined in 1% agarose gels stained with SYPRO Safe (1:100000; Invitrogen) and analysed using Quantity One software (Bio-Rad).

### 2.5. Sample preparation for proteomics analysis

The frozen liver tissue samples from the four groups were homogenized and lysed in a lysis buffer (pH 7.5) comprising 1% Triton X-100 (USB Corporation, USA), 25 mmol/L HEPES (Sigma-Aldrich), 150 mmol/L NaCl (UNIVAR, USA), 1 mmol/L EDTA disodium salt (Sigma-Aldrich), 1 mmol/L dithiothreitol (DTT) (USB Corporation), and 1% Protease Inhibitor Cocktail Set III (Bio-Rad). The lysis samples were kept on ice for 40 minutes and then centrifuged at 12,000× g for 30 minutes at 4 °C. To remove the superfluous salt in the extract, 150 μL of the supernatants were mixed with 600 μL of methanol by vortexing. In total, 150 μL of chloroform and 450 μL of ultrapure water were added in turn; the samples were vortexed to mix well. The mixtures were centrifuged at 12,000× g for 5 minutes. The upper phase was discarded and the white precipitation disc was kept. Then, 450 μL of methanol was added and mixed well; the protein pellet was obtained by centrifugation at 12,000× g for 5 min at 4 °C. After air-drying, the protein pellet was resuspended in buffer (pH 8.0) comprised of 7 mol/L urea (USB Corporation), 2 mol/L thiourea (Sigma-Aldrich), 100 mmol/L DTT, 5% glycerol (USB Corporation), and 4% CHAPS (Sigma-Aldrich). The resulting protein solution was stored at –80 °C until the 2-DE analysis. The protein concentration was determined by the Bradford assay (Bio-Rad).

### 2.6. 2-DE procedures

The 2-DE procedures were performed according to our previous study with several modifications (Jiang et al., 2008; Wang et al., 2012). The liver tissue samples were processed in sextuplicate, and a total of 24 gels were used. For the first-dimension electrophoresis, 150-μg protein samples were mixed with 350 μL of rehydration buffer comprised of 9.5 mol/L urea, 2% CHAPS, 0.28% DTT, 1% immobilized pH gradient buffer with pH 3–10 (Bio-Rad), and 0.002% bromophenol blue (Sigma-Aldrich). The samples were then applied to an Ettan IPGphor3 isoelectric focusing electrophoresis system (GE Healthcare, USA). The samples were rehydrated for 10 h prior to isoelectric focusing using the following programmes: (a) linear increase up to 500 V over 1 h; (b) holding at 500 V for 2 h; (c) linear increase up to 1000 V over 4 h; (d) linear increase up to 10,000 V over 3 h; and (e) final hold at 10,000 V to reach a total of 90,000 V × h. The focused immobilized pH gradient gel strips equilibrated for 15 min in a solution comprised of 50 mmol/L Tris–HCl at pH 8.8, 6 mol/L urea, 30% glycerol, 2% sodium dodecyl sulphate (SDS) (USB Corporation), and 20 mmol/L DTT. This process was followed by an incubation step with the identical buffer containing 20 mmol/L iodoacetamide (Sigma-Aldrich) for another 15 min. The second-dimension separation was performed in 1-mm-thick pH 8.8 12.5% polyacrylamide gels by SDS-PAGE. The run profile was a constant current of 30 mA for 30 min followed by a 60-mA current for the remainder of the analysis until the bromophenol blue line arrives at the bottom of the gels.

### 2.7. Image acquisition and analysis

After the 2-DE, the gels were stained with SYPRO Ruby Protein Stain (Bio-Rad) according to the manufacturer's protocol. The stained gels were scanned with a Molecular Imager PharosFX Plus System (Bio-Rad) and analysed by PDQuest 8.0 software (Bio-Rad). Each expression level was calculated as the percentage volume (% vol) and exported for statistical analysis. The relative intensities of spots were used for comparison between the two groups, and only those spots with significant differences (greater than 1.5-fold increase or decrease; $p < 0.05$) were selected for protein identification.

### 2.8. Protein identification

Spots showing differential expression ($p < 0.05$) between the EVOO fed and corn oil fed animals with $CCl_4$-treatment were sent to the Genome Research Centre (The University of Hong Kong, Hong Kong) for protein identification. The proteins were digested with trypsin and applied to a matrix-assisted laser desorption/ionization-time-of-flight/time-of-flight MS (MALDI-TOF/TOF MS) apparatus using a 4800 MALDI TOF/TOF Analyzer (Applied Biosystems, USA). Matches between the experimental data and mass values calculated from a candidate protein were performed by Mascot

Please cite this article in press as: Hualin Wang, Wat-Hung Sit, George Lim Tipoe, Jennifer Man-Fan Wan, Differential protective effects of extra virgin olive oil and corn oil in liver injury: A proteomic study, Food and Chemical Toxicology (2014), doi: 10.1016/j.fct.2014.09.002

Download English Version:

https://daneshyari.com/en/article/5849963

Download Persian Version:

https://daneshyari.com/article/5849963

Daneshyari.com