Toxicology Letters 232 (2015) 233–245

Contents lists available at ScienceDirect

Toxicology Letters

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

iTRAQ-based proteomic analysis of combination therapy with taurine, epigallocatechin gallate, and genistein on carbon tetrachloride-induced liver fibrosis in rats

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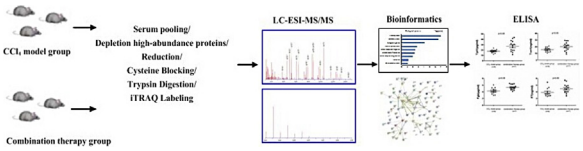
HIGHLIGHTS

- iTRAQ-based proteomic analysis of combination therapy in liver fibrosis rats.
- Identified differentially expressed proteins and bioinformatics analysis.
- Protein content of Tpi1, Txn1, Fgb, and F7 were validated by ELISA.
- Antioxidant system and glycolysis pathway may be the mechanisms of combination therapy.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form 3 November 2014
Accepted 10 November 2014
Available online 13 November 2014

Keywords:
Epigallocatechin gallate
Genistein
iTRAQ
Liver fibrosis
Proteomic
Taurine

ABSTRACT

Combination therapy with taurine, epigallocatechin gallate, and genistein was effective in alleviating the progression of liver fibrosis in our previous study. To better understand the anti-fibrotic mechanisms of combination therapy, an iTRAQ-based proteomics approach was used to study the expression profiles of proteins in carbon tetrachloride-induced liver fibrosis rats following combination therapy. The anti-fibrotic effects of combination therapy were assessed directly by liver histology, and indirectly by measurement of serum biochemical markers and antioxidant enzymes. The results showed that combination therapy could significantly improve the liver function, as indicated by decreasing levels of alanine aminotransferase (ALT), aspartate transaminase (AST), transforming growth factor-β1 (TGF-β1), and collagen I, increasing levels of total antioxidative capacity (T-AOC), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), and reducing the pathological tissue damage. A total of 89 differential expressed proteins in response to combination therapy were identified by iTRAQ, which were interacted with each other and involved in different biological processes and pathways. Four differentially expressed proteins (Tpi1, Txn1, Fgb, and F7) involved in antioxidant defense system, glycolysis pathway

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BP, biological process; CC, cellular component; CCl_4, carbon tetrachloride; DAVID, database for annotation, visualization, and integrated discovery; ECM, extracellular matrix; EGCG, epigallocatechin gallate; ELISA, enzyme-linked immunosorbent assay; Fgb, fibrinogen beta chain; F7, coagulation factor VII; GSH-Px, glutathione peroxidase; iTRAQ, isobaric tags for relative and absolute quantitation; MF, molecular function; Mst4, serine/threonine-protein kinase Mst4; PVDF, polyvinylidene fluoride; ROS, reactive oxygen species; SCX, strong cation exchange; SOD, superoxide dismutase; T-AOC, total antioxidative capacity; TEAB, tetraethylammonium bromide; TGF-β1, transforming growth factor-β1; Tpi1, triosephosphate isomerase; Txn1, thioredoxin.

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http://dx.doi.org/10.1016/j.toxlet.2014.11.009
0378-4274/

and coagulation cascade pathway were validated by enzyme-linked immunosorbent assay. Our work provided valuable insights into the molecular mechanism of combination therapy against liver fibrosis, and the identified targets may be useful for treatment of liver fibrosis in future.

1. Introduction

Liver fibrosis is a wound-healing process, which can lead to the subsequent development of cirrhosis and eventually hepatocellular carcinoma if not treated at the early stage. Many studies have been shown that liver fibrosis is reversible whereas cirrhosis, the end-stage consequence of fibrosis, is generally irreversible (Bonis et al., 2001; Geramizadeh et al., 2008). Thus, there is an urgent need to develop medications for treatment of liver fibrosis.

Many distinct triggers can contribute to the development of liver fibrosis, involving complicated factors that require multiple therapies. Obviously, effects of monotherapy with either antioxidants or tyrosine kinase inhibitors are limited (Distler and Distler, 2010; Sánchez-Valle et al., 2012; Campo et al., 2004), and a higher dosage of a single drug would have more side effects (Gebhardt, 2002). In this regard, it is essential to provide a therapy with higher efficiency and fewer adverse effects for the treatment of liver fibrosis. Combination therapies with multiple drugs are superior to monotherapy due to their synergistic effects and the reduction of side effects (Carragher et al., 2012; Kumar and Sarin, 2008). Moreover, they can target various sites of action and interfere in different stages of fibrogenesis. Therefore, combination therapies may potentially provide a powerful therapy for treatment of liver fibrosis.

Over the past years, accumulating evidence suggests that taurine, epigallocatechin gallate (EGCG), and genistein, which have different therapeutic targets, are effective against liver fibrosis. Taurine can inhibit the expression of transforming growth factor-β1 (TGF-β1) and block the TGF-β 1/Smad pathway thereby promoting the apoptosis of HSC (Chen et al., 2004; Kato et al., 2004; Miyazaki et al., 2005). EGCG has therapeutic potential due to its powerful antioxidant properties that can inhibite collagen production and suppress collagenase activity (Nakamuta et al., 2005). As a tyrosine kinase inhibitor, genistein can suppress proliferation and activation of HSC (McCarty et al., 2009). We have previously reported that the combination therapy with taurine, EGCG, and genistein can inhibit HSC proliferation, affect the expression of fibrosis-related gene and protein in vitro more effectively than monotherapy ($P < 0.05$) (Li et al., 2013). Furthermore, our previous study also revealed that, compared with monotherapy, combination therapy can effectively restrain the serum levels of fibrosis markers and inhibit the deposition of collagen, the expression of α-smooth muscle actin, B-cell lymphoma 2 and Smad3 so as to attenuate alcohol-induced liver fibrosis in rats ($P < 0.05$) (Zhuo et al., 2012). Therefore, we believe that combination therapy will provide a more effective therapy for the treatment of liver fibrosis than monotherapy. However, the mechanisms of combination therapy against liver fibrosis are still not well understood at the molecular level. So, a more thorough analysis at proteome levels is required to clarify the mechanisms of protective effect against liver fibrosis of combination therapy.

Advancements in the field of proteomics have made it possible to accurately monitor and quantitatively detect the changes of protein expression in response to drug treatment, and have been used to investigate the mechanisms of chemicals on liver diseases (Lee et al., 2009; Wang et al., 2012). iTRAQ is a robust mass spectrometry technology that allows quantitative comparison of protein abundance by measuring peak intensities of reporter ions released from iTRAQ-tagged peptides by fragmentation during MS/MS (Niu et al., 2009). The iTRAQ-based technology has been widely applied in quantitative proteomic applications because of its ever-greater specificity, selectivity, and sensitivity. This technology has been successfully used to identify the differentially expressed proteins in liver fibrosis (Yang et al., 2011; Zhang et al., 2011).

In the present paper, we employed iTRAQ combined with LC-ESI–MS/MS analyses to investigate the protective effect and the possible anti-fibrotic mechanism of combination therapy on CCl_4-induced liver fibrosis rats, and applied western blot and ELISA for further confirmation. With these measurements, we aimed to identify protein profile changes in response to liver fibrosis with the potential mechanistic relevance to combination therapy. We found that glycolysis pathway, antioxidant defense and blood coagulation system were the most significantly up-regulated biological process under combination therapy. These results provided valuable insights into the molecular mechanism of combination therapy against liver fibrosis.

2. Materials and methods

2.1. Animals and experimental design

Thirty-five Sprague–Dawley rats (weighing 200–250 g, SPF) were obtained from Experimental Animal Center of Guangxi Medical University (Guangxi, China). The research was conducted according to protocols approved by the institutional ethical committee of Guangxi Medical University. The rats were housed under controlled conditions with temperature of 25 ± 2 °C, relative humidity of 60 ± 10%, and a 12/12 light–dark cycle with free access to water and a standard rat diet.

After a period of one week, rats were randomly separated in two groups: a CCl_4-induced liver fibrosis group ($n = 26$) and a normal control group ($n = 9$). The CCl_4 group received CCl_4 (Guangdong Guanghua Sci-Tech Co., Ltd., Guangdong, China) 2 mL/kg diluted in peanut oil twice a week by intragastric administration for 8 weeks in order to establish the rat model of liver fibrosis, and the normal control group was given the same volume of peanut oil until the end of the experiment. In order to monitor the formation of liver fibrosis, histological examinations of liver were performed on 2 rats of the CCl_4-induced liver fibrosis group and 1 rat of the normal control which were randomly drawn out at the 6–8th week.

After the rat model of liver fibrosis had been established, the 13 survival liver fibrosis rats were randomly divided into two subgroups: CCl_4 model group ($n = 6$), in which rats received the same volume of saline; and combination therapy group ($n = 7$), in which rats received combination therapy (taurine 100 mg/kg + EGCG 15 mg/kg + genistein 10 mg/kg) by intragastric administration (taurine: 2-aminoethanesulfonic acid, $C_2H_7NO_3S$, Chengdu Kelong Chemical Reagent Factory, Sichuan, China; EGCG: (2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate, $C_{22}H_{18}O_{11}$, Leshan Yujia Tea Science and Technology Development Co., Ltd., Sichuan, China; genistein: 4′,5,7-trihydroxyisoflavone, $C_{15}H_{10}O_5$, Meryer Chemical Technology Co., Ltd., Shanghai, China).

All rats were anesthetized and sacrificed after 6 weeks treatment. Serum samples were collected and stored at −80 °C until used for further determination. Liver was sectioned and fixed in 10% formalin solution for histological examination.