Environmental Toxicology and Pharmacology 45 (2016) 170–178

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

Betaine treatment decreased oxidative stress, inflammation, and stellate cell activation in rats with alcoholic liver fibrosis

İlknur Bingül[a], Canan Başaran-Küçükgergin[a], A.Fatih Aydın[a], Jale Çoban[b], Işın Doğan-Ekici[c], Semra Doğru-Abbasoğlu[a,*], Müjdat Uysal[a]

[a] Department of Biochemistry, Istanbul Medical Faculty, Istanbul University, Çapa, Istanbul, Turkey
[b] Department of Biochemistry, Yeditepe University Medical Faculty, Kayışdağı, Istanbul, Turkey
[c] Department of Pathology, Yeditepe University Medical Faculty, Kayışdağı, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form 30 May 2016
Accepted 31 May 2016
Available online 1 June 2016

Keywords:
Betaine
Alcoholic liver fibrosis
Oxidative stress
Stellate cell activation
Rat

ABSTRACT

The aim of this study was to investigate the effect of betaine (BET) on alcoholic liver fibrosis in rats. Fibrosis was experimentally generated with ethanol plus carbon tetrachloride (ETH + CCl_4) treatment. Rats were treated with ETH (5% v/v in drinking water) for 14 weeks. CCl_4 was administered intraperitoneally (i.p.) 0.2 mL/kg twice a week to rats in the last 6 weeks with/without commercial food containing BET (2% w/w). Serum hepatic damage markers, tumor necrosis factor-α, hepatic triglyceride (TG) and hydroxyproline (HYP) levels, and oxidative stress parameters were measured together with histopathologic observations. In addition, α-smooth muscle-actin (α-SMA), transforming growth factor-β1 (TGF-β1) and type I collagen (COL1A1) protein expressions were assayed immunohistochemically to evaluate stellate cell (HSC) activation. mRNA expressions of matrix metalloproteinase-2 (MMP-2) and its inhibitors (TIMP-1 and TIMP-2) were also determined. BET treatment diminished TG and HYP levels; prooxidant status and fibrotic changes; α-SMA, COL1A1 and TGF-β protein expressions; MMP-2, TIMP-1 and TIMP-2 mRNA expressions in the liver of fibrotic rats. In conclusion, these results indicate that the antifibrotic effect of BET may be related to its suppressive effects on oxidant and inflammatory processes together with HSC activation in alcoholic liver fibrosis.

1. Introduction

The "two-hit" theory is a widely accepted mechanism for the development and progression of alcoholic liver disease (ALD). Steatosis is the first lesion in ALD and may progress to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Banerjee et al., 2013; Szabo and Mandrekar, 2010). It is accepted that the accumulation of fat in the liver predisposes the liver to secondary stresses such as oxidative stress, inflammation, and cytokines (Banerjee et al., 2013; Szabo and Mandrekar, 2010). Although liver fibrosis is reversible, cirrhosis and HCC are generally irreversible (Hernandez-Gea and Friedman, 2011). Therefore, one of the main therapeutic strategies may be preventing the progression of steatosis to fibrosis and other advanced hepatic lesions. For this reason, the preventive potential of several antioxidants on alcoholic liver fibrosis development has been tested (Banerjee et al., 2013; Ramaiah et al., 2004).

Betaine (BET), also known as trimethyl glycine (Fig. 1), an oxidative metabolite of choline, has been known to be involved in the synthesis of methionine from homocysteine in the liver. It acts as an osmolyte and has antioxidant and anti-inflammatory effects (Day and Kempson, 2016; Ueland et al., 2005). Previous studies have shown that BET protects the liver against various hepatotoxic agents. Indeed, BET treatment was shown to be useful in alcoholic (Balkan et al., 2004; Jung et al., 2013) and non-alcoholic fatty liver (Wang et al., 2010), as well as in necrotic (Balkan et al., 2005) and fibrotic (Erman et al., 2004; Kim et al., 2009; Tsai et al., 2015) liver lesions. However, the effects of BET on alcoholic liver fibrosis development and related mechanisms are still unknown.

Fibrosis is an excessive wound healing response to chronic liver injury characterized by the accumulation of an extracellular matrix (ECM) rich in fibrillar collagens, mainly type I and III collagen. Activated hepatic stellate cell (HSC) is the main fibrogenic cell. HSC activation is crucial in liver fibrogenesis (Hernandez-Gea and Friedman, 2011).

* Corresponding author.
E-mail address: sdabbasoglu@yahoo.com (S. Doğru-Abbasoğlu).

http://dx.doi.org/10.1016/j.etap.2016.05.033
1382-6689/

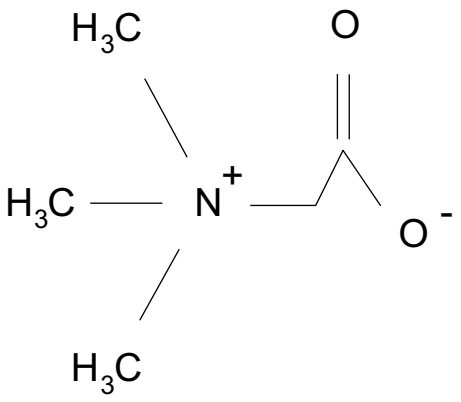

Fig. 1. The structure of betaine.

In this study, an alcoholic liver fibrosis model was created trough the administration of ethanol (ETH) together with low doses of carbon tetrachloride (CCl_4). We aimed to examine the effects of BET treatment on the development of fibrosis and related mechanisms. Accordingly, we measured the serum levels of hepatic damage markers and tumor necrosis factor-α (TNF- α). Triglyceride (TG) and hydroxyproline (HYP) levels and oxidative stress parameters were measured in the liver together with histopathologic observations. To evaluate HSC activation, we also assayed α-smooth muscle-actin (α-SMA), transforming growth factor-β1 (TGF-β1), and type I collagen (COL1A1) protein expressions, as well as mRNA expressions of matrix metalloproteinase-2 (MMP-2) and its inhibitors (TIMP-1 and TIMP-2).

2. Materials and methods

2.1. Chemicals

BET was supplied from Alfa Aesar (Germany) and other chemicals were purchased from Sigma-Aldrich (St Louis, Missouri, USA). All reagents were of analytical grade.

2.2. Animals

Female Sprague Dawley rats weighing 180–200 g were used in the study. They were obtained from the Experimental Medical Research Institute of Istanbul University. Rats were housed in a light- and temperature-controlled room on a 12/12-h light/dark cycle. The animals had free access to food and water and were kept in wire-bottomed stainless steel cages. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of the Istanbul University.

2.3. Experimental design

In this study, the effect of BET treatment was investigated in alcoholic fibrosis model. Rats were treated with ethanol for 14 weeks. CCl_4 was administered to rats in the last 6 weeks with or without BET containing diet. Accordingly, rats were randomly selected and divided into four groups as follows:

1. Control group: Rats were fed with commercial rat chow and injected i.p. with an olive oil vehicle (twice a week for the last 6 weeks).
2. BET group: Rats were fed with normal commercial food for 8 weeks and then 2% w/w BET-containing commercial food for 6 weeks together with i.p. olive oil vehicle injections twice a week.
3. ETH + CCl_4 group: Rats were fed with normal commercial food and treated with ETH in the drinking water with increasing concentrations; 1% ETH (v/v) for the first two days, 2% from day 3 until the end of the 1st week, 4% in the second week, and 5% for another 12 weeks. CCl_4 (i.p. 0.2 mL/kg diluted 1:4 in olive oil) was also administered twice a week to rats in the last 6 weeks.
4. ETH + CCl_4 + BET group: Rats were treated with ETH + CCl_4 as described above. Rats were fed with BET (2% w/w)-containing commercial food in the last 6 weeks.

2.4. Blood and tissue samples

At the end of the treatment period, all rats were sacrificed by taking blood via cardiac puncture under sodium thiopental anesthesia (50 mg/kg, i.p.). Blood was collected in dry tubes. Livers were rapidly removed, washed in 0.9% NaCl and kept in ice. Serum samples were obtained by centrifugation at 1500*g* for 10 min. The materials were stored at −80 °C until required for analysis. Liver tissue was homogenized in ice-cold 0.15 M KCl (10%; w/v) and homogenates were centrifuged at 600*g* for 10 min and this fraction was used for biochemical determinations in the liver.

2.5. Determinations in serum

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activity measurements were performed on a Cobas Integra 800 autoanalyzer (Roche Diagnostics, Mannheim, Germany). TNF-α (Rat TNF-alpha, Diaclone SAS, Besancon, France) levels were measured using commercial enzyme-linked immunosorbent assay ELISA kits.

2.6. Determinations in liver

2.6.1. Determination of TG levels

Hepatic lipids were extracted with chloroform:methanol (2:1) (Folch et al., 1957) and TG levels were determined in lipid extracts using enzymatic procedures.

2.6.2. Determination of HYP levels

Hepatic HYP levels were measured using the method of Bergman and Loxley (1963). In brief, 100 mg of liver tissue was hydrolyzed in 4 mL of 6 N HCl at 108 °C for 16 h in a glass tube with a Teflon stopper. The hydolysate was filtered and neutralized with 6 N NaOH. This solution was then pipetted into a clean tube and incubated with oxidizing reagent containing chloramines-T. Ehrlich's reagent was added to the tube and incubated at 60 °C for 45 min. Absorbances of samples and standards were read at 570 nm and the results were calculated using a standard curve.

2.6.3. Determinations of reactive oxygen species (ROS) generation, malondialdehyde (MDA,) and protein carbonyl (PC) levels

ROS generation was fluorometrically assayed as described previously (Wang and Joseph, 1999). Liver homogenates were incubated with 100 μM 2′,7′-dichlorodihydrofluorescein diacetate at 37 °C for 30 min. The fluorescence of 2′,7′-dichlorofluorescein was determined using a microplatefluorometer (Fluoroskan Ascent FL, Thermo scientific Inc, USA) with an excitation of 485 nm and emission of 538 nm. Results were expressed as relative fluorescence units (RFU).

Lipid peroxidation was determined by measuring MDA levels according to Ohkawa et al. (1979). 1,1,3,3-tetraethoxypropane was used as a standard. Results were expressed as nmol/mg protein.

The oxidative protein damage was measured spectrophotometrically. This method is based on the reaction between 2,4-dinitrophenylhydrazine and PC to form protein hydrazones.