European Journal of Pharmacology 689 (2012) 194–203

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Pulmonary, gastrointestinal and urogenital pharmacology

Effect of diethylcarbamazine on chronic hepatic inflammation induced by alcohol in C57BL/6 mice

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ARTICLE INFO

Article history:
Received 10 January 2012
Received in revised form
31 May 2012
Accepted 31 May 2012
Available online 7 June 2012

Keywords:
Diethylcarbamazine
Alcoholism
Inflammatory markers
Hepatic injury

ABSTRACT

Some pharmacological studies showed that diethylcarbamazine (DEC) interferes with the arachidonic acid metabolism, acting as an anti-inflammatory drug. The chronic alcohol consumption activates the hepatic inflammatory response associated to T-cell activation and overproduction of pro-inflammatory cytokines. The present work analyzed the anti-inflammatory effect of DEC on hepatic cells of alcoholic mice. Thirty-two male C57BL/6 mice were equally divided in the following groups: (a) control group (C), which received only water, (b) DEC-treated group, which received 50 mg/kg for 12 day (DEC50), (c) the alcoholic group (EtOH), submitted to only alcohol and (d) the alcohol-DEC treated group (EtOH50), submitted to alcohol plus DEC treatment after the induction of chronic alcoholism for 5 weeks. Biochemical analyses were performed and liver fragments were processed for light microscopy, transmission electron microscopy, immunohistochemical and western blot. The level of AST increased significantly in alcoholic group whereas a significant reduction of serum AST was detected in the EtOH50 group. Histological and ultrastructural analysis of alcoholic group showed evident hepatocellular damage, which was strikingly reduced in the alcoholic DEC-treated group. Immunohistochemistry results revealed highly expression of inflammatory markers as MDA, NF-κB, TNF-α, IL-6, VCAM and ICAM by the hepatic cells of the EtOH group; however no immunoreactivity for any of these cytokines was detected after DEC treatment. Western blot analyses showed increased MCP-1 and iNOS expression in EtOH group, which was significantly inhibited by DEC treatment. According to the present results, DEC can be a potential drug for the treatment of chronic inflammation induced by chronic alcoholism.

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1. Introduction

The harmful use of alcohol is a serious health burden, and it affects virtually all individuals on an international scale. Health problems from dangerous alcohol use arise in the form of acute and chronic conditions, and adverse social consequences are common when they are associated with alcohol consumption. Every year, the harmful use of alcohol kills 2.5 million people, including 320 000 young people between 15 and 29 years of age. Alcohol abuse and alcoholism represents one of the major health, social and economic issues facing the world (WHO, 2011). Liver is the major site of ethanol metabolism, and thus the most susceptible organ to alcohol induced injury (Kannarkat et al., 2006). Alcoholic liver disease (ALD) is a collective term for the pathophysiological changes caused by chronic alcohol consumption, which include oxidative stress generation, liver steatosis, inflammatory response, fibrosis and cirrhosis. Progression of the disease involves various proinflammatory molecules such as interleukins, cytokines, adhesion molecules and nuclear factor-κB (NF-κB) (Nanji et al., 1999; French, 2002; Achur et al., 2010; Ballas et al., 2012). The activated NF-κB, if being translocated into the nucleus, will facilitate transcription of many downstream genes, including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2); both are key mediators in recruitment of inflammatory cells (Bhaskaran et al., 2010; Arias-Salvatierra et al., 2011). The iNOS-derived nitric oxide production is activated downstream of NF-κB, followed by generation of reactive oxygen species and other free radicals that are detrimental to cells. Cellular lipids are easily attacked by free radicals, resulting in intracellular

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0014-2999/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2012.05.044

accumulation of malondialdehyde (MDA) (Chen et al., 2011). The marked generation of oxidative stress associated with ethanol metabolism is one of the main liver injuries caused by chronic alcohol consumption. Oxidative stress causes dysfunctions in several cellular mechanisms, such as DNA repair and antioxidant systems (Bardag-Gorce et al., 2000).

Several pharmaco-therapeutic studies have been undertaken to cure alcoholic hepatitis. The best known are the treatments that block tumor necrosis factor α (TNF-α) and reduce inflammation (pentoxifylline, infliximab, etanercept) (Akriviadis et al., 2000; Naveau et al., 2004; Boetticher et al., 2008). However, these treatments are associated with an increase of infections and death, because of this treatment of alcohol-induced liver disease remains limited to supportive measures (Mullen and Dasrathy, 1998; Barve et al., 2008). Undoubtedly, the development of effective therapy to prevent or treat ALD will depend on elucidating the suppression/blockage of any of the steps culminating into liver injury.

The diethylcarbamazine is the drug most widely used in the treatment of lymphatic filariasis since 1947 (Hewitt et al., 1947). Some studies performed on vertebrates show that this drug has several direct biochemical effects on different enzyme systems, including glycolysis, folate metabolism and activity of acetylcholinesterase (Subrahmanyam, 1987). DEC also has anti-inflammatory properties as a result of its interference with the arachidonic acid metabolism, which includes lipoxygenases (LOX) and cyclooxygenases (COX) enzymes (Maizels and Denham, 1992; McGarry et al., 2005). In studies carried out in association with our laboratory, Queto et al. (2010) demonstrated that DEC has important role in blocking the pulmonary eosinophilic inflammation in mice sensitized with ovalbumin, effectively preventing the effects of subsequent airway resistance, Th1/Th2 cytokine production, pulmonary eosinophil accumulation and eosinophilopoiesis in vivo and *ex vivo*. Besides, DEC directly suppressed IL-5-dependent eosinophilopoiesis in naive bone marrow.

In this study, for the first time, was demonstrated that DEC can decrease liver injury in experimental alcoholism model, and revealed a clinical potential of DEC for therapeutic anti-inflammatory applications.

2. Material and methods

2.1. Animals

Thirty-two male C57BL/6 mice at 5 weeks old and weighting 15–16 g were used in all experiments. Mice were examined for health status and acclimated to the laboratory environment at 23 °C and 12 h light: 12 h dark photoperiod. The animals were housed in metal cages and fed a standard diet and water ad libitum. The animal studies Ethics Committee of Oswaldo Cruz Institute approved all the experiments reported.

2.2. Diethylcarbamazine solutions

The solutions were compound of distilled water and DEC (Farmanguinhos, FIOCRUZ, Brazil) adjusted according to the body weight of each animal. The groups that were treated received 50 mg/kg of DEC for twelve days through gavage (200 μl) (Saraiva et al., 2006; Rocha et al., 2012). The control group (C) received just distilled water by the same administration via. Classical metabolic studies describes the total metabolic rate of a 30 g mouse as 961 kJ per kg body weight, which is approximately seven times the total metabolic rate of a 70 kg human, 138 kJ per kg (Terpstra, 2001). The lymphatic filariasis therapeutic dose regimens recommended by WHO is 6 mg/kg for 12 day (WHO, 2011). In the present work, we used 50 mg/kg of DEC for 12 day, which is about seven times the human dose.

2.3. Experimental groups

After a week of acclimation, C57BL/6 mice with 30 day were separated in four groups ($n=8$): (1) Control group (C) that received just distilled water by the same administration via, (2) DEC-treated group (D50) that received 50 mg/kg DEC for twelve days by gavage, (3) alcoholic group (EtOH) that received ethanol and (4) alcoholic plus 50 mg/kg DEC group (EtOH50). Ethanol was provided in the drinking water at 10% (v/v) for 2 day, 15% for 5 day, and 20% for 5 weeks (Cook et al., 2004; Ballas et al., 2012). According to Cook et al. (2004), the mice innate immune system presents significant changes after 3 weeks of 20% ethanol consumption. The solutions were changed daily and the remaining liquid in the bottles was measured in graduated cylinder for final volume analysis and then discarded. After 5 weeks of alcoholism induction, mice received alcohol plus treatment for 12 day with DEC solutions orally. The alcoholic group received alcohol plus water by gavage for 12 day.

2.4. Biochemical determinations

Serum was evaluated for the lipid profile (total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride) and hepatic damage test (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirrubin). Serum concentrations were measured by spectrophotometer method in Integra 400– Roche. Data were compared by ANOVA one way and Dunnet's or Tukey tests used GraphPad Prism software, version 5.

2.5. Histopathology

Liver fragments were fixed in 10% formalin for 24 h, processed and embedded in paraffin. Sections $4-5$ μm were cut, mounted on glass slides. Slices were stained with hematoxylin-eosin and evaluated with an inverted microscopy (Observer Z1, Zeiss MicroImaging GmbH) equipped with a camera and 4.7.4 image analysis program (AxionCam MRm Zeiss) at a magnification of 400 ×.

2.6. Electron transmission microscopy

The fragments of liver were fixed in a solution containing 2.5% glutaraldehyde and 4% formaldehyde in 0.1 M cacodylated buffer. After fixation, the samples were washed twice in the same buffer, and then they were post-fixed in a solution containing 1% osmium tetroxide, 2 mM calcium chloride and 0.8% potassium ferricyanide in 0.1 M cacodylate buffer, pH 7.2, dehydrated in acetone, and embedded in Epon 812 resin (Sigma Company, St Louis, MO). Polymerization was done at 60 °C for 2 day. Ultrathin sections were collected on 300-mesh copper grids, counterstained with uranyl acetate and lead citrate, and examined with a Morgani FEI transmission electron microscope.

2.7. Immunohistochemical assays

Five sections (5 μm in thickness) of each group were cut and adhered to slides treated with 3-amino-propyl-trietoxi-silane (APES [Sigma, USA]). Briefly, sections were deparaffinized with xylene and rehydrated in graded ethanol (100% to 70%). To increase epitope exposure, the sections were heated for 30 min in a sodium citrate buffer (0.01 M, pH 6.0). To minimize endogenous peroxidase activity, the slides were treated with 0.3% (v/v)

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