Toxicology and Applied Pharmacology 311 (2016) 1–11

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Carvedilol suppresses circulating and hepatic IL-6 responsible for hepatocarcinogenesis of chronically damaged liver in rats

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ARTICLE INFO

Article history:
Received 18 July 2016
Revised 7 October 2016
Accepted 11 October 2016
Available online 13 October 2016

Keywords:
Carvedilol
IL-6
E & N-cadherin
CCL_4
Hepatic cirrhosis
Hepatocellular cancer

ABSTRACT

Carvedilol is an anti-oxidant non-selective β-blocker used for reduction of portal blood pressure, prophylaxis of esophageal varices development and bleeding in chronic liver diseases. Recently, it exhibited potent anti-inflammatory, anti-fibrotic, anti-proliferative and anti-carcinogenic effects. In the present study, we evaluated the possible suppressive effect of carvedilol on circulating and hepatic IL-6 levels responsible for hepatocarcinogenesis in a rat model of hepatic cirrhosis. Besides, its effect on hepatic STAT-3 levels, function tests, oxidative stress markers, and hydroxyproline content, hepatic tissue histopathological changes and immunohistochemical expression of E & N-cadherin. Nine-week-old male Wistar rats injected intraperitoneal by 1 ml/kg 10% CCL_4 in olive oil three times/week (every other day) for 12 weeks to induce hepatic cirrhosis. Carvedilol (10 mg/kg/day suspended in 0.5% CMC orally), silymarin (50 mg/kg/day suspended in 0.5% CMC orally) or combination of both used to treat hepatic cirrhosis from 15th to 84th day. Our data showed that carvedilol and silymarin co-treatment each alone or in combination efficiently reduced the elevated serum IL-6, ALT, AST, ALP and BIL, hepatic IL-6, STAT-3, MDA levels and hydroxyproline content. In addition, it elevated the reduced serum ALB level, hepatic CAT activity and GSH level. Meanwhile, it apparently restored the normal hepatic architecture, collagen distribution and immunohistochemical E & N-cadherin expression. Furthermore, carvedilol was superior to silymarin in improving MDA level. Moreover, the combination of carvedilol and silymarin showed an upper hand in amelioration of the CCL_4 induced hepatotoxicity than each alone. Therefore, carvedilol could be promising in prevention of hepatocarcinogenesis in chronic hepatic injuries.

1. Introduction

Chronic liver diseases such as viral hepatitis, steatohepatitis, chronic alcoholism and autoimmune disorders are a worldwide health problem with catastrophic sequelae whatever the etiology. One of these sequels is hepatic cirrhosis, which ends eventually into hepatocellular carcinoma (HCC) in about 5% of cirrhotic patients. HCC is a slowly developed cancer that needs several decades to be established, and accounts for >85% of all primary liver cancer. It considered as the fifth commonest and the second deadly worldwide carcinoma, with an expected lifespan of 4–6 months at the time of detection (Mittal and El-Serag, 2013; Marengo et al., 2016).

Interleukin (IL)-6 is one of the most pivotal pro-inflammatory cytokines, which play an important role in liver regeneration (Wen et al., 2015). However, persistent activation of IL-6 trans-signaling pathway is tumorigenic and able to induce malignant transformation of chronically inflamed hepatic tissue (Jung et al., 2015; Schmidt-Arras and Rose-John, 2016). In the early stage of hepatic inflammation, the elevated level of circulating IL-6 secreted from leukocytes rather than hepatic parenchymal cells (Soresi et al., 2006). Soon after, the elevated level of IL-6 activates Kupffer cells with M_2 phenotype polarization, which augment the IL-6 secretion (Mauer et al., 2014). The augmented paracrine level of IL-6 enhances the development of dysplastic hepatic foci containing HCC progenitor cells (He et al., 2013). Evidences suggest that, HCC progenitor cell development is through IL-6 activation of the oncogenic transcription factor signal transducer and activator of transcription (STAT)-3, nuclear factor kappa B (NFκB) and extracellular signal-regulated kinase (ERK)-1 (Weber et al., 2003; Jin et al., 2011; Jung et al., 2015). In late stages of hepatic inflammation, HCC progenitor cells attain an autocrine function due to upregulation of LIN28 expression, and secrete IL-6, which enhances further progression of malignant transformation and tumor growth (He et al., 2013).

Evidence of recent prospective study of obese patient with different metabolic disorder interconnected the elevated serum IL-6 level with high risk of HCC development (Aleksandrova et al., 2014). In addition, the elevated level of circulating IL-6 considered as a bad prognostic criterion in chronic viral hepatitis and predisposing to HCC development (Wong et al., 2009). Moreover, the elevated serum levels of IL-6 and sIL-6R were detected in HCC patients (Soresi et al., 2006). In contrary,

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http://dx.doi.org/10.1016/j.taap.2016.10.012
0041-008X/

HCC development is reduced greatly in IL-6 knockout mice and in female mice, as estrogen suppresses IL-6 secretion and activity, via activation of estrogen receptor-α (Naugler et al., 2007).

Nonselective β-blockers are a commonly used medication in the treatment regime of hepatic fibrosis and cirrhosis, aiming for a reduction of portal blood pressure, prevention of esophageal varices development and bleeding, and avoidance of other longstanding complications for instance ascites, hepatocellular failure and renal dysfunction. Propranolol is still the gold standard β-blocker used in chronic hepatic disorder, however, recent studies showed that carvedilol is better than propranolol in reducing hepatic venous pressure (Abid et al., 2015).

Carvedilol is one of non-selective β-blockers used clinically for the treatment of hypertension, angina pectoris, small myocardial infarction, tachyarrhythmia and congestive heart failure. It resolves these conditions not only by its β-blocking activities, but also due to its vasodilating effect; through α_1 blocking action, and potent anti-oxidant properties (Keating and Jarvis, 2003). Moreover, carvedilol proved to possess powerful anti-inflammatory, anti-fibrotic, anti-proliferative and anti-carcinogenic effects (Stanojkovic et al., 2005; Hamdy and El-Demerdash, 2012; Hakucho et al., 2014). Furthermore, carvedilol treatment suppresses the production of pro-inflammatory cytokines as IL-6 and TNF-α in idiopathic dilated cardiomyopathy (Kurum et al., 2007). All of these potentialities favor its usage in chronic liver diseases to prevent and ameliorate hepatic cirrhosis induced by various offending agents.

Therefore, in the present study, we evaluated the possible suppressive effect of carvedilol on circulating and hepatic IL-6 levels, incriminated in hepatocarcinogenesis, in a rat model of hepatic cirrhosis. As well as its effect on hepatic STAT-3 levels, function tests, oxidative stress markers, and hydroxyproline content, hepatic tissues histopathological changes and immunohistochemical expression of E and N-cadherin.

2. Materials and methods

2.1. Drugs and reagents

All drugs and reagents were obtained commercially as follow; carbon tetrachloride (CCL_4), pyridine, sulfuric acid, Ellman's reagent, chloramine-T, Ehrlich's reagent, L-hydroxyproline, Bouin's solution, phosphotungstic acid, acid fuchsine, aniline blue & orange G (Sigma, St. Louis, MO, USA), carvedilol (Multi Pharma, Nasr city, Cairo, Egypt), silymarin (Medical Union Pharmaceutical, Nasr city, Cairo, Egypt), methanol, carboxy methyl cellulose (CMC), phosphate buffer, phosphate-buffered saline (PBS), hydrogen peroxide, Tris-HCl buffer, formalin buffered saline, sodium dodecyl sulfate, acetic acid, sodium hydroxide (NaOH), oxalic acid, hematoxylin and eosin stains (El Gomhuria Co., Tanta, El-Gharbeya, Egypt), sodium pentobarbitone (Abbott Lab., Chicago, IL, USA), thiobarbituric acid (Riedel-de Haën, AG., Germany), and N-butanol & 1,1,3,3 tetramethoxypropane (VWR International Ltd., Ballycoolin, Dublin, Ireland).

2.2. Animals

The current study was performed using nine-week-old male Wistar rats, weighing 160–200 g, obtained from Tanta University animal house, and allowed to acclimatize for one week before starting the experiment. All animals were accommodated in plastic cages at a temperature of 22 ± 2 °C, with relative humidity of 60 ± 10%, exposed to a 12-hour light/dark cycle, and fed a standard laboratory diet and water ad libitum. All experiments carried out following the guideline for the care and use of experimental animals in Faculty of Medicine, Tanta University, Egypt, with an approval of Animal Experiment Ethics Committee of the Faculty.

2.3. The experimental design

Rats were randomly divided into seven groups of 10 rats each. Group I (control) was normal rats. Group II (olive), normal rat received 1 ml/kg olive oil intraperitoneally (ip) three times/week (every other day) for 12 weeks. Group III (CCL), hepatic cirrhosis group induced by ip injection of 1 ml/kg 10% CCL_4 in olive oil three times/week (every other day) for 12 weeks (Lee et al., 2005). Group IV (CMC), hepatic cirrhosis group treated orally with 1 ml/kg/day 0.5% CMC in PBS from 15th to 84th day. Group V (SIL), hepatic cirrhosis group treated orally by silymarin 50 mg/kg/day suspended in 0.5% CMC from 15th to 84th day (Lin et al., 2012). Group VI (CAR), hepatic cirrhosis group treated orally by carvedilol 10 mg/kg/day suspended in 0.5% CMC from 15th to 84th day (Hakucho et al., 2014). Group VII (SIL + CAR), hepatic cirrhosis group treated orally by silymarin 50 mg/kg/day and carvedilol 10 mg/kg/day suspended in 0.5% CMC from 15th to 84th day. All suspensions were prepared fresh daily. In the days of CCL_4 injection, all treatments were received 1 h after the inoculation.

2.4. Sample collection

Twenty-four hours after the last treatment, rats were anesthetized by ip injection of 50 mg/kg pentobarbital sodium, followed by blood collection through the retro-orbital puncture. The collected blood centrifuged at 5000 rpm for 10 min, sera collected and stored at −30 °C for further evaluation of serum parameters. Immediately after blood collection, rats scarified by cervical dislocation, laparotomy were done, livers harvested and washed with ice-cold PBS. Then, 1 g of hepatic tissues were resected and homogenized in 10 ml of 0.1 mM Tris-HCl buffer (pH = 7). The homogenate divided into two equal amounts, one of them centrifuged at 5000 rpm for 10 min and the supernatant collected. The supernatants and the remaining homogenates stored at −30 °C for further assessment of hepatic tissue parameters. The rest of hepatic tissues were processed according to the method described by Suvarna et al. (2013). Shortly, liver specimens immediately fixed in 10% formalin buffered saline, dehydrated, impregnated, embedded in hard paraffin, sectioned into 5 μm sections and used for histopathological and immunohistochemical examination.

2.5. Evaluation of serum and hepatic IL-6 levels

The serum and hepatic IL-6 levels were evaluated according to the manufacturer's instructions of enzyme-linked immunosorbent assay (ELISA) Kits obtained from Abnova Co., Taipei, Taiwan, with minimal detection limit 12 pg/ml. Optical densities were assessed and analyzed using an automated plate reader (Stat Fax 2100, Fisher Bioblock Scientific, BP., Illkirch Cedex, France). Serum IL-6 levels expressed as pg/ml, however hepatic IL-6 levels expressed as pg/g hepatic tissue.

2.6. Evaluation of hepatic STAT-3 levels

The hepatic STAT-3 levels were estimated according to the manufacturer's instructions of a sandwich enzyme-linked immunosorbent assay (sELISA) Kits obtained from AVIVA Systems Biology, Beijing, China, with minimal detection limit 0.076 ng/ml. Optical densities were assessed and analyzed using an automated plate reader (Stat Fax 2100, Fisher Bioblock Scientific, BP., Illkirch Cedex, France). Hepatic STAT-3 levels expressed as ng/g hepatic tissue.

2.7. Evaluation of hepatic function tests

Hepatic function tests were assessed using a colorimetric assay kits obtained from Bio-Diagnostic Co., Dokki, Giza, Egypt. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels according to the method described by Reitman and Frankel (1957), serum alkaline phosphatase (ALP) level according to the method designated by