Cytokine 75 (2015) 127–135

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

PPARα/γ agonists and antagonists differently affect hepatic lipid metabolism, oxidative stress and inflammatory cytokine production in steatohepatitic rats

Yan Zhang [a,b], Yan Cui [a], Xiao-Li Wang [a], Xiang Shang [a], Zhi-Gang Qi [c], Jie Xue [a], Xi Zhao [a,d], Min Deng [e], Mei-Lin Xie [a,*]

[a] Department of Pharmacology, Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, Jiangsu Province, PR China
[b] Department of Gynecology and Obstetrics, Wuxi Maternal and Child Health Hospital, The Affiliated Hospital of Nanjing Medical University, Wuxi 214002, Jiangsu Province, PR China
[c] Department of Pharmacy, Wuxi People's Hospital, Wuxi 214023, Jiangsu Province, PR China
[d] Department of Pharmaceutical Laboratory, Medical College of Nantong University, Nantong 226001, Jiangsu Province, PR China
[e] Department of Pathology, Medical College of Soochow University, Suzhou 215123, Jiangsu Province, PR China

ARTICLE INFO

Article history:
Received 24 October 2014
Received in revised form 22 April 2015
Accepted 26 May 2015
Available online 17 July 2015

Keywords:
Non-alcoholic steatohepatitis
Peroxisome proliferator-activated receptor alpha/gamma
Lipid metabolism
Oxidative stress
Inflammatory cytokines

ABSTRACT

Peroxisome proliferator-activated receptor (PPAR) α/γ may control lipid metabolism and inflammatory response by regulating the downstream target genes, and play a crucial role in the process of non-alcoholic steatohepatitis (NASH) formation, but the difference and interaction between PPARα and PPARγ are poorly understood. The rat model with NASH was established by orally feeding high-fat and high-sucrose emulsion for 6 weeks. The results shown that after the model rats were simultaneously treated with PPARα/γ agonists, the total cholesterol (TC), triglyceride (TG) and inflammatory cytokine levels in serum and hepatic tissue, the hepatic steatosis and inflammatory cellular infiltration were decreased, and were consistent with the results of hepatic lipogenic gene and nuclear factor (NF)-κB protein expressions. Conversely, these indexes were increased by PPARα/γ antagonist treatment. Compared with the model group, the serum free fatty acid (FFA) level was increased in the PPARα agonist-treated group, decreased in the PPARγ agonist-treated group, and unchanged in the PPARα/γ agonists-treated group. The hepatic FFA level was low in the PPARα/γ agonists-treated groups, but no significant variation in the PPARα/γ antagonists-treated groups. The increments of hepatic reduced glutathione (GSH) and superoxide dismutase (SOD) contents in the PPARα/γ agonists-treated groups were accompanied by decreased hepatic malondialdehyde (MDA) content. These findings demonstrated that PPARα/γ activation might decrease the hepatic lipid accumulation, oxidative stress and inflammatory cytokine production, and PPARγ could counterbalance the adverse effect of PPARα on circulating FFA. It was concluded that the integrative application of PPARα and PPARγ agonists might exert a synergic inhibitory effect on NASH formation through the modulation of PPARα/γ-mediated lipogenic and inflammatory gene expressions.

1. Introduction

The cardinal feature of non-alcoholic steatohepatitis (NASH) is hepatic lipid accumulation and inflammatory cellular infiltration, this disease has increasingly been recognized as a precursor to more severe liver diseases including hepatic fibrosis, cirrhosis and hepatocellular carcinoma [1]. Although the prevalence of NASH appears to be increasing, the exact pathogenesis remains poorly understood [1,2]. However, the "two-hit" hypothesis proposed by Day et al. is widely accepted as the pathogenesis of NASH [3]. The first hit is from hepatic lipid accumulation, which may mainly be due to the increment of triglyceride (TG) synthesis per se in liver and/or decrement of TG-rich lipoprotein secretion into systemic circulation [4,5]. The second hit is proposed to be a multifactorial process, including oxidative stress, lipid peroxidation, nuclear factor kappa B (NF-κB) activation and related pro-inflammatory cytokine production. These insults may finally

* Corresponding author at: Department of Pharmacology, College of Pharmaceutical Sciences, Soochow University, 199 Renai Road, Suzhou Industrial Park, Suzhou 215123, Jiangsu Province, PR China.
E-mail address: xiemeilin@suda.edu.cn (M.-L. Xie).

http://dx.doi.org/10.1016/j.cyto.2015.05.031
1043-4666/

lead to inflammatory cellular infiltration and contribute to the progression of simple steatosis to steatohepatitis [6].

Abnormal lipid metabolism in liver is the basis of NASH formation. Recent studies have demonstrated that free fatty acid (FFA) plays an important role in the development of NASH [7,8]. The hepatic excessive fatty acids may derive from the high-fat diets, hepatic de novo lipogenesis and adipose tissue lipolysis. Under normal physiological condition, the hepatic fatty acids can either be oxidized to generate ATP or esterified to produce TG. The latter can be incorporated into TG-rich lipoprotein for export, and the reduction of the lipoprotein secretion may result in the hepatic lipid accumulation and steatosis. On the other hand, the hepatic fatty acid overloading may also promote the oxidation itself and reactive oxygen species (ROS) generation [2,9], and result in the imbalance between oxidants and antioxidants, which appears to be responsible for initiating necroinflammation and considered a key phenomenon in the progression from simple steatosis to steatohepatitis.

Indeed, ROS and lipid peroxidation are the factors that induce inflammation through the production of pro-inflammatory cytokines and subsequent inflammatory cellular infiltration [10,11]. Several documents have demonstrated that ROS may trigger the activation of NF-κB inflammatory pathway in liver [12–14], which is up-regulated both in rodent models and in patients with NASH [15,16]. In fact, NF-κB is the master transcription factor in the control of inflammatory response [14,17,18], and it may increase the synthesis and release of tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), IL-8 and monocyte chemoattractant protein-1 (MCP-1) [2,11]. The simple fatty liver is susceptible to these inflammatory cytokines and chemokines, which may further induce liver injury and steatohepatitic formation [19].

Peroxisome proliferator-activated receptor (PPAR) α/γ are nuclear receptors that may control the lipid metabolism by regulating the expressions of lipogenic genes, such as sterol regulatory element binding protein (SREBP)-1c, fatty acid synthase (FAS), diacylglycerol acyltransferase (DGAT) and lipoprotein lipase (LPL) [20–23]. PPARα is predominantly expressed in the liver, and its expression may decrease hepatic lipid accumulation and protect mice from high fat-induced hepatic steatosis [24,25]. PPARγ is present in the liver and adipose tissue, and its activation plays a major role in increasing insulin sensitivity [26]. In addition, PPARα/γ can also ameliorate inflammatory response by inhibition of NF-κB activation and reduction of related inflammatory cytokine and chemokine release [27–29]. PPARα agonists may protect the liver from obesity-induced hepatic inflammation and reverse steatohepatitis in mice [30,31], while PPARγ is more specific for controlling NF-κB-mediated inflammatory response [32].

These literature data reveal that PPARα/γ may involve in the process of two hits of NASH formation, but the difference and interaction between PPARα and PPARγ are incompletely understood yet. In this study, we used the specific PPARα/γ agonists and antagonists to probe their interaction and different effects on hepatic lipid metabolism, oxidative stress and inflammatory cytokine production in steatohepatitic rats.

2. Materials and methods

2.1. Drugs and reagents

Fenofibrate and rosiglitazone were procured from Laboratories Fournier SA (Chenove, France) and Chengdu Hengrui Pharmaceutical Co., Ltd. (Chengdu, China), and were suspended in 0.5% sodium carboxymethyl cellulose solution, respectively. MK886 (purity > 99%) and GW9662 (purity > 98%) were purchased from Cayman Chemical Company (Michigan, USA) and dissolved in 5% dimethylsulfoxide (DMSO) solution. The assay kits for total cholesterol (TC) and TG were purchased from Beijing Beihua Kangtai Clinical Reagent Company (Beijing, China). The assay kits for FFA, reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The ELISA kits for rat TNF-a, IL-6, IL-8 and MCP-1 were purchased from Shanghai Xitang Bio-technology Co., Ltd. (Shanghai, China). The commercial kit for protein extract was supplied by Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China), and bicinchoninic acid kit for protein concentration assay was supplied by Beyotime Institute of Biotechnology (Jiangsu, China). Anti-PPARα and anti-DGAT antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-FAS and anti-SREBP-1c antibodies were products of Abcam (Cambridge, UK). Anti-LPL antibody was obtained from Novus Biologicals (Littleton, CO, USA). Anti-PPARγ, anti-NF-κB and anti-β-actin antibodies were purchased from Cell Signaling Technology Company (Boston, USA). All other reagents used in this study were of analytical grade.

2.2. Animals

Male Sprague–Dawley rats (200 ± 20 g) were supplied by the Experimental Animal Center of Soochow University, housed in regular cages in a room controlled temperature (20 ± 1 °C) and humidity (60% ± 10%), and allowed free access to food and water. The animals were allowed to acclimatize to the laboratory environment for 3 days prior to the study. All animal studies were approved by the University Ethic Committee and conducted according to the regulations for the use and care of experimental animals at Soochow University.

2.3. Establishment of high-fat and high-sucrose-induced steatohepatitic model

The experimental rats were randomly divided into eight groups ($n = 8$): control group, model group, PPARα agonist (fenofibrate 20 mg/kg) group, PPARγ agonist (rosiglitazone 4 mg/kg) group, fenofibrate 20 mg/kg plus rosiglitazone 4 mg/kg group, PPARα antagonist (MK886 1 mg/kg [33]) group, PPARγ antagonist (GW9662 1 mg/kg [34]) group and MK886 1 mg/kg plus GW9662 1 mg/kg group. The PPARα/γ agonists-treated rats were orally given fenofibrate and/or rosiglitazone by gavage and intraperitoneally given 5% DMSO solution, the PPARα/γ antagonists-treated rats were intraperitoneally given MK886 and/or GW9662 and orally given 0.5% sodium carboxymethyl cellulose solution, and the control and model rats were given 0.5% sodium carboxymethyl cellulose and 5% DMSO solution in the same manner in the morning for 6 weeks. All drugs or vehicles were administered in a constant volume of 0.2 mL/100 g body weight. The steatohepatitic model was simultaneously induced by orally feeding a high-fat and high-sucrose emulsion at 1 mL/100 g body weight once per day in the afternoon for 6 weeks [35]. The control rats were treated with an equivalent volume of distilled water by gavage daily. Finally, all of the rats were sacrificed, blood and liver were collected for parameter measurements, and partial hepatic tissues were snap-frozen in liquid nitrogen and stored at −80 °C for Western blot assay.

2.4. Measurement of TC, TG and FFA contents in serum and hepatic tissue

Rat blood was obtained after 12 h of overnight fasting. Partial livers were collected and homogenized (10%, w/v) in cold normal saline. The tissue homogenate was then mixed with a solution of chloroform/methanol (2:1, v/v) according to a ratio of 1:1 (v/v).