Journal of Pharmacological Sciences 127 (2015) 430–438

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

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

Full paper

High-fat diet enhanced retinal dehydrogenase activity, but suppressed retinol dehydrogenase activity in liver of rats

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A R T I C L E I N F O

Article history:
Received 23 October 2014
Received in revised form
28 February 2015
Accepted 2 March 2015
Available online 7 March 2015

Keywords:
Hyperlipidemia
Retinoid
Alcohol dehydrogenases
Retinol dehydrogenases
Retinaldehyde dehydrogenases

A B S T R A C T

Evidence has shown that hyperlipidemia is associated with retinoid dyshomeostasis. In liver, retinol is mainly oxidized to retinal by retinol dehydrogenases (RDHs) and alcohol dehydrogenases (ADHs), further converted to retinoic acid by retinal dehydrogenases (RALDHs). The aim of this study was to investigate whether high-fat diet (HFD) induced hyperlipidemia affected activity and expression of hepatic ADHs/RDHs and RALDHs in rats. Results showed that retinol levels in liver, kidney and adipose tissue of HFD rats were significantly increased, while plasma retinol and hepatic retinal levels were markedly decreased. HFD rats exhibited significantly downregulated hepatic ADHs/RDHs activity and *Adh1*, *Rdh10* and *Dhrs9* expression. Oppositely, hepatic RALDHs activity and *Raldh1* expression were upregulated in HFD rats. In HepG2 cells, treatment of HFD rat serum inhibited ADHs/RDHs activity and induced RALDHs activity. Among the tested abnormally altered components in HFD rat serum, cholesterol reduced ADHs/RDHs activity and *RDH10* expression, while induced RALDHs activity and *RALDH1* expression in HepG2 cells. Contrary to the effect of cholesterol, cholesterol-lowering agent pravastatin upregulated ADHs/RDHs activity and *RDH10* expression, while suppressed RALDHs activity and *RALDH1* expression. In conclusion, hyperlipidemia oppositely altered activity and expression of hepatic ADHs/RDHs and RALDHs, which is partially due to the elevated cholesterol levels.

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1. Introduction

The prevalence of obesity is becoming a threatening risk to human health. Obesity, characterized by severe hyperlipidemia and insulin resistance (1), is one of the potential risk factors of non-insulin dependent diabetes mellitus (NIDDM). It is generally accepted that obesity mainly results from the combined effects of excess energy intake and reduced energy expenditure (2). In addition to energy, dietary nutrients provide us essential vitamins and other factors with regulatory roles. However, the effects of individual micronutrients on the development of metabolic diseases are not fully understood.

Vitamin A (retinol) is an essential fat-soluble micronutrient involved in multiple crucial functions, such as vision, tissue differentiation and immunity (3–5). The physiological functions of retinol are mainly mediated via its active metabolite, retinoic acid (RA). RA regulates gene expression through activating nuclear receptors (6) including retinoic acid receptors (RAR) and retinoid X receptors (RXR). Recent studies have shown that retinoids participate in glucose and lipid metabolism and adipogenesis via activating RAR and RXR (7–9).

Liver plays important roles in the homeostasis of vitamin A via the transport, storage, production, and metabolism of retinoids (10,11). In liver, the dietary retinol is first oxidized to retinal by both cytosolic alcohol dehydrogenases (ADHs) of the medium-chain dehydrogenase/reductase superfamily and microsomal retinol dehydrogenases (RDHs) of the short-chain dehydrogenase/reductase superfamily, subsequently oxidized to RA by retinal dehydrogenases (RALDHs). The conversion of retinal into RA is irreversible and considered to be the rate-limiting step in RA biosynthesis (3,12,13). Some reports have demonstrated that hepatic ADHs/RDHs and RALDHs play important roles in the maintenance of retinoid homeostasis (14–16), which indicates that the alterations in activity of ADHs, RDHs and RALDHs may impact on physiological functions via affecting RA formation. A report showed

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Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2015.03.001
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that *Rdh1* null mice exhibited increased size and adiposity (14), while *Raldh1*-knockout mice were protected from diet-induced obesity and insulin resistance (17). Treatment of retinal or RALDHs inhibitor reduced fat deposit and increased insulin sensitivity of ob/ob mice (17). *Raldh1*-knockout mice were reported to exhibit attenuated gluconeogenesis and lower fasting glucose level, which resulted from the downregulated expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase 1 (18).

Obesity is often associated with retinol disorder. Several clinical investigations validate that obesity is frequently accompanied by retinol inadequacy (19–22), which is considered to be associated with the increased inflammatory response in obesity (23). As we mentioned before, ADHs/RDHs and RALDHs play significant roles in the regulation of retinol levels. However, it remains unclear whether obesity could change the expression and activity of hepatic ADHs/RDHs and RALDHs and, in turn, lead to retinol disorder.

The aim of the study was: firstly to investigate whether dyshomeostasis of retinoids occurred in rats with hyperlipidemia induced by high-fat diet (HFD); secondly to investigate the effect of hyperlipidemia on the activity and expression of hepatic ADHs/RDHs and RALDHs; thirdly to identify components affecting ADHs/RDHs and RALDHs activity in the serum of hyperlipidemia rats using HepG2 cells as an *in vitro* model.

2. Material and methods

2.1. Chemicals and reagents

All-trans retinol, all-trans retinaldehyde, all-trans retinoic acid (atRA), cholesterol, insulin, palmitic acid, oleic acid, stearic acid, linoleic acid, O-Ethylhydroxylamine hydrochloride, β-Nicotinamide adenine dinucleotide sodium salt (β-NAD), β-Nicotinamide adenine dinucleotide phosphate sodium salt (β-NADP) and streptozotocin (STZ) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bovine serum albumin (BSA) was purchased from Qcbio Science & Technologies Co., Ltd. (Shanghai, China). 2-Nitrophenylhydrazine hydrochloride (2-NPH·HCl) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-hydrochloride (1-EDC·HCl) were purchased from J&K Chemical, Ltd. (Beijing, China). All the other reagents were of analytical grade and were commercially available.

2.2. Animals and induction of hyperlipidemia rats

Male Sprague–Dawley rats, weighing 100–110 g, were purchased from SIPPR/BK Experimental Animal Co. Ltd. (Shanghai, China). A total of 20 rats were employed in this study. The rats were maintained under controlled conditions of temperature (22 ± 2 °C) and relative humidity (50 ± 10%) with 12-h light–dark cycle. They were allowed free access to food and water. The study was approved by Animal Ethics Committee of China Pharmaceutical University (NO.CPU-PCPK-S1010354).

Hyperlipidemia rats were induced by feeding HFD according to previous methods (24–27). The rats were acclimated for three days before the experiment and divided randomly into normal control (CON) group and HFD group. The HFD (Xietong, Nanjing, Jiangsu) consisted of 15% lard (w/w), 5% sesame oil, 20% sucrose, 2.5% cholesterol and 57.5% normal chow for 8 weeks. Both HFD and normal chow contained standard vitamin A. One batch of rats (n = 5 for each group) were fed with HFD or normal chow for 8 weeks. During this period, blood samples were collected once a week via the oculi chorioideae vein of rats under light ether anesthesia after 6 h fasting, fasting blood glucose (FBG) concentration was determined using a glucose reagent kit (Jiancheng Biotech Co., Nanjing, China), body weight and energy intake were recorded daily. On week 8, the experimental rats were fasted overnight and sacrificed under light ether anesthesia. Blood and tissues (liver, adipose tissue, kidney) were immediately collected for the determination of biochemical parameters and retinoid concentration. Parts of liver were used for measuring biochemical parameters and retinoid concentration. The remaining liver was used for preparing hepatic microsomes and cytosol, assessing levels of targeted gene mRNA. Insulin, triglyceride (TG), total cholesterol (TC) and free fatty acids (FFA) were determined on week 8. Plasma insulin was measured by iodine [^{125}I] insulin RIA kit (BNIBT Co., Beijing, China). Plasma and hepatic TG and TC levels were measured by test kits (BHKT Clinical Reagent, Beijing, China) using 10 μl plasma and 10% liver homogenate, respectively. The concentrations of free palmitic acid, strearic acid, leinoleic acid and oleic acid in plasma were assayed as their 2-nitrophenylhydrazine derivatives by HPLC method previously described (28).

2.3. Retinol pharmacokinetics in experimental rats

Another batch of rats (n = 5 for each group) were fed with HFD or normal chow for 8 weeks. On week 8, these rats were fasted overnight and were orally administrated retinol (intragastric gavage 45 mg/kg, dissolved in olive oil) according to a previous report (29). Blood samples (about 0.25 ml) were collected into heparized tubes via the oculi chorioideae vein under light ether anesthesia at 0, 1, 2, 3, 5, 7, 9, 12, 24 and 36 h post dose of retinol. The plasma samples were stored at −80 °C for retinoid analysis.

2.4. Activity of ADHs/RDHs and RALDHs in hepatic cytosol and microsomes of rat

Hepatic cytosol and microsomes were prepared freshly from experimental rats according to the method previously described (30). The protein concentrations of the cytosol and microsomes were measured by Bradford method using BSA as the standard (31).

Activity of ADHs/RDHs and RALDHs in hepatic cytosol and microsomes were assessed according to previously described method with minor modifications (32). Briefly, hepatic ADHs/RDHs and RALDHs activity was evaluated with retinal formation and all-trans atRA formation in the presence of β-nicotinamide adenine dinucleotide (β-NAD$^+$) and β-Nicotinamide adenine dinucleotide phosphate (β-NADP$^+$), respectively. The incubation mixture consisted of hepatic cytosol/microsomes (at the final concentration of 0.5 mg/ml), and a series concentrations of retinol or retinal, 4.0 mM co-factor, 5 mM $MgCl_2$, 91 μM butylated hydroxytoluene in a total volume of 500 μl. The reaction was initiated by the addition of retinol or retinal pre-incubated for 5 min at 37 °C. Following 15 min incubation, the reaction was quenched by extraction with equal volume of n-butanol/methanol, 95:5 (v:v) containing 0.005% butylated hydroxytoluene. Organic phase was collected after centrifugation and stored at −80 °C for analyses (33).

2.5. Effect of hyperlipidemia rat serum on ADHs/RDHs and RALDHs activity in HepG2 cells

The serum of experimental rats was inactivated by heating at 56 °C for 30 min following filtering through 0.22 -μm filter. HepG2 cells were treated with DMEM supplemented with 10% serum for 24 h (34). Briefly, sub-confluent (approximately 80%) HepG2 cells were incubated with DMEM containing 10% serum from CON and HFD rats for 24 h. Then, HepG2 cells were collected for the preparation of S9-mixtures according to the protocol previously described (35,36). The ADHs/RDHs and RALDHs activity in S9-mixtures was carried out according to the method described above using NAD$^+$ as cofactor.

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