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The effects of estradiol valerate and remifemin on liver lipid metabolism

Biao Jin[a,1], Wenjuan Wang[a,1], Wenpei Bai[b], Jing Zhang[a], Ke Wang[a], Lihua Qin[a,*]

[a] Department of Anatomy & Histology and Embryology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China
[b] Department of Obstetrics and Gynecology, Beijing Shijitan Hospital, Capital Medical University, Beijing 100038, China

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ABSTRACT

To investigate the lipid metabolism dysregulation in the liver of ovariectomized (OVX) rats and effects of estradiol valerate (E) and remifemin (ICR) thereon, forty female Sprague-Dawley rats were randomly divided into sham-operated (SHAM), OVX, OVX + E, and OVX + ICR group. After 4 weeks' E or ICR treatment, serum estrogen, cholesterol, and triglyceride levels; lipid droplets in hepatocytes; hepatocyte morphology; and the expression of estrogen receptor α (ERα), liver X receptor (LXR), and sterol regulatory element binding proteins (SREBPs) in the liver of the rats were assessed. OVX rats had significantly decreased serum estrogen levels, which significantly increased after treatment with E but not with ICR. Serum triglyceride levels and the amount of lipid droplets in hepatocytes increased after ovariectomy, and significantly decreased after E treatment. In addition, ICR treatment markedly increased serum triglyceride levels and lipid droplet size. No significant differences in the serum cholesterol levels were observed among the four groups. After ovariectomy, hepatocyte mitochondria became hypertrophic and misformed, which were reversed with E or ICR treatment. ICR-treated rats also showed endoplasmic reticulum disorganization. After ovariectomy, ERα and LXR levels significantly decreased while SREBP expression increased. E treatment increased ERα and LXR levels while ICR treatment only increased LXR expression. E treatment decreased SREBP-1c levels, whereas SREBP-1c levels increased with ICR treatment. Treatment with E significantly reversed the ovariectomy-induced dysregulation of hepatocyte lipid metabolism, which was, however, exacerbated with ICR treatment. The effects of E and ICR on hepatocyte lipid metabolism may involve the regulation of LXR and SREBP-1c.

1. Introduction

Perimenopausal obesity is common in women undergoing involutional changes due to the decrease in estrogen levels, which can alter lipid metabolism. Therefore, weight gain is one of the principal health problems in women aged 55–65 years (Nappi and Kokot-Kierepa, 2012). Generally, the prevalence of obesity in women is higher than that in men; however, the underlying reason remains unclear. Changes in the levels of sex hormones during different physiological stages such as menarche, pregnancy, and menopause affect tissue fat deposition. For example, several studies have shown that during the perimenopausal period, the deposition of abdominal adipose tissue increases (Davis et al., 2012). Perimenopausal obesity not only influences lipid metabolism but can also cause cardiac-related cerebrovascular disease, hypertension, and diabetes (Polotsky and Polotsky, 2010). Therefore, investigating the mechanisms underlying altered liver lipid metabolism in obese perimenopausal women as well as potential drug therapies targeting these mechanisms is important.

Estrogen is an important hormone with extensive physiological functions. Estrogen receptors (ERs) are steroid hormone receptors that modulate the physiological functions of estrogen (Weihua et al., 2000; Krege et al., 1998; Fan et al., 1999; Campbell-Thompson et al., 2001). ERs are expressed abundantly and comprise three subtypes: estrogen receptor α (ERα), estrogen receptor β (ERβ), and G protein-coupled estrogen receptor (GPER). ERα is mainly expressed in the liver, an organ playing an essential role in lipid metabolism (Mowa and Iwanaga, 2000; Denger et al., 2001). The distribution of ERα and ERβ in adipocytes varies between different organs. Further, only ERα is known to inhibit fat deposition (Heine et al., 2000; Naaz et al., 2002). Some studies have shown that estradiol valerate (E) can effectively influence food intake in mammals. In addition, E plays an important role in maintaining lipid and glucose homeostasis. E has been shown to

Abbreviations: ERs, estrogen receptors; ERα, estrogen receptor α; ERβ, estrogen receptor β; ERE, estrogen response element; E, estradiol valerate; GPER G, protein-coupled estrogen receptor; ICR, Remifemin; LH, luteinizing hormone; LXRs, liver X receptors; NIH, National Institutes of Health; OVX + E, OVX with E treatment; OVX + ICR, OVX with ICR treatment; SREBP-1c, sterol regulatory element binding protein-1c; TG, triglyceride; TC, total cholesterol; TEM, transmission electron microscopy

* Corresponding author.
E-mail address: qinlihua88@163.com (L. Qin).
[1] These authors contributed equally to this work.

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reduce serum triglyceride levels in mice fed a high-fat diet (Faulds et al., 2012; D'Eon et al., 2005; Hewitt et al., 2004; Bryzgalova et al., 2008; Gao et al., 2006); however, the exact underlying mechanism is not completely understood. Other research has shown that ERs can inhibit lipoprotein lipase during the fasting state, and that an increase in serum estrogen levels can decrease the synthesis of triglycerides, as well as reduce the elongation of long-chain fatty acids family member 6 and fatty acid synthase factors (Iverius and Brunzell, 1988; Urabe et al., 1996). These factors play an important role in the aliphatic acid biosynthesis pathway.

Liver X receptors (LXRs), which were discovered in 1994 (Willy et al., 1995), can regulate cholesterol absorption, synthesis, and excretion. There are two homologous LXR subtypes: LXRα and LXRβ. LXRα is not only highly expressed in the liver and involved in lipid metabolism, but is also found in adipose tissue, the prostate, intestines, lungs, kidneys, and macrophages (Zelcer and Tontonoz, 2006). LXRβ, on the other hand, is expressed throughout the body (Zelcer and Tontonoz, 2006). LXRs increase the expression of sterol regulatory element binding protein-1c (SREBP-1c), resulting in activation of lipogenesis (Horton et al., 2002). SREBPs are membrane-bound transcription factors, which participate in several aspects of lipid homeostasis in the liver (Ou et al., 2001). For example, SREBP-1c plays an essential role in the synthesis of triglycerides and phospholipids, whereas SREBP-2 mainly promotes the synthesis of cholesterol. The transcription of SREBP-1c can be activated by LXRs; therefore, inhibiting LXRs can downregulate the expression of SREBP-1c mRNA (Zhang et al., 2001). SREBP-1c can also be activated by insulin and inhibited by glucagon (Shimomura et al., 2000).

Remifemin (ICR) is the isopropanolic extract of the rhizome of the North American herb black cohosh (*Cimicifuga racemosa* (L.) Nutt. [CR], also known as raceme cohosh). Each tablet of ICR contains 1 mg of triterpene as the active ingredient, extracted from 20 mg of the crude drug. Black cohosh belongs to the Ranunculaceae family and grows in the eastern part of North America. It is used as a medicinal plant in Europe for more than a century. Black cohosh can be taken in normal doses for up to 6 months without showing any known side effects. Therefore, it is extensively used as an alternative for estrogen to treat perimenopausal problems. There is limited evidence that black cohosh may cause hepatic failure (Levitsky et al., 2005). The National Institutes of Health (NIH) reported that the results from a clinical trial on black cohosh did not confirm hepatotoxicity. Nevertheless, the NIH suggested that liver function should be monitored during clinical trials of black cohosh. Other research showed that black cohosh extract did not affect body weight or other clinical parameters; however, at doses of 667 and 2000 mg/kg it increased liver weight and serum alanine transaminase activity in women (Yun et al., 2015).

ERs affect liver lipid metabolism in rats (Faulds et al., 2012). For example, estrogen levels decreased and lipid metabolism was altered accordingly in ovariectomized (OVX) rats (Han et al., 2014). At present, there are conflicting data regarding the therapeutic effect of ICR, and it is unknown whether the effects of ICR and E are mediated through common biological pathways. Therefore, in this study, we investigated whether and how ICR affects liver lipid metabolism. In addition, the effects of ICR were compared to those of E. We found that both E and ICR affected liver lipid metabolism through SREBP-1c. However, whether LXRα may play a role in this pathway remains unknown.

2. Materials and methods

2.1. Laboratory animals

Forty adult female Sprague-Dawley (SD) rats aged 9–10 weeks were obtained from the Department of Laboratory Animal Science of Peking University Health Science Center. All of the experiments were approved by the Laboratory Animal Welfare Ethics Committee of Peking University Health Science Center (approval number: LA2012-82). The rats were acclimatized for 1 week before the experiments and were fed a particle chow without beans (to exclude any potential effects of phytoestrogens) until the end of the experiments. The room temperature was kept at 25 °C, with a relative humidity of 50–55% and a 12-h/12-h light/dark cycle. The rats were allowed access to food and water *ad libitum* during the experiments.

2.2. Laboratory reagents and instruments

Mouse anti-rat LXRα (ab41902) was purchased from Abcam (Abcam, Cambridge, UK), rabbit anti-rat Phospho-SREBP-1c (Ser372) was purchased from CST, and rabbit anti-rat ERα antibody (SC-542) and the immunohistochemical ABC kit (SP-9001, SP-9002, PV6002) were purchased from Beijing Zhongshan Golden Bridge Biological Technology Co., Ltd. (Beijing, China). ICR was purchased from Schaper & Brümmer GmbH & Co. KG (batch number 063471; Salzgitter, Germany). Each ICR tablet contained 20 mg of the crude drug, which was extracted with 40% isopropyl alcohol to yield an average of 2.5 mg of dried extract. E (1 mg active drug per tablet) was obtained from Bayer HealthCare Co., Ltd. (batch number 026A11; Guangzhou, China). Further, an ultrathin microtome (model 1900; Leica Biosystems, Wetzlar, Germany) and an Olympus BX51 microscope (Olympus, Tokyo, Japan) were used in this study.

2.3. Ovariectomized rat model

Forty rats were randomly divided into 4 groups: a SHAM, OVX, OVX with E treatment (OVX + E), and OVX with ICR treatment (OVX + ICR) group. Each group contained 10 rats. All of the rats, except those in the SHAM group, were bilaterally ovariectomized under aseptic conditions. Briefly, anesthesia was induced with an intraperitoneal injection of 1% pentobarbital sodium (40 mg/kg) and a median abdominal incision of a length of 2–3 cm was made. The abdominal cavity was opened and the uterus was ligated using a forceps and filament. The ovaries were removed and the incision was sealed. The rats in the SHAM group underwent sham surgery. Briefly, after anesthesia induction with 1% pentobarbital, the pelvic cavity was opened without subsequent removal of the ovaries. The incisions were sealed thereafter. Starting from the third day after ovariectomy, vaginal smears were collected for up to 10 days to assess whether the surgery had been successful.

2.4. Drug administration

Two weeks after being ovariectomized, the rats were administered the respective drugs by oral gavage between 8:00 AM and 9:00 AM each day for four weeks. The SHAM and OVX groups were administered 10 mL/kg normal saline. The OVX + E rats were administered 0.8 mg/kg E and the OVX + ICR rats were administered 60 mg/kg ICR. The doses of E and ICR were adjusted according to the daily body weight, and were prepared as previously reported (Xiao-yan et al., 2011).

2.5. Serology

Serum samples were collected from all of the rats in the four groups and the triglyceride (TG) and total cholesterol (TC) were measured using a Hitachi 7600-100 clinical analyzer (Roche, Japan, Department of Laboratory Medicine, Peking University Third Hospital, Beijing, China). Serum estrogen levels were measured using a radioimmunoassay (RIA) kit (R066, Solarbio Science and technology co.,ltd, Beijing). Testing steps were carefully carried out in accordance with the instructions in it.