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Serum lipidomics analysis of ovariectomized rats under *Curcuma comosa* treatment

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ABSTRACT

Ethnopharmacological relevance: *Curcuma comosa* Roxb. (*C. comosa*) or Wan Chak Motluk, Zingiberaceae family, has been used in Thai traditional medicine for the treatment of gynecological problems and inflammation.
Aim of the study: This study aimed to investigate the therapeutic potential of *C. comosa* by determining the changes in the lipid profiles in the ovariectomized rats, as a model of estrogen-deficiency-induced hyperlipidemia, after treatment with different components of *C. comosa* using an untargeted lipidomics approach.
Materials and methods: Lipids were extracted from the serum of adult female rats subjected to a sham operation (SHAM; control), ovariectomy (OVX), or OVX with 12-week daily doses of estrogen (17β-estradiol; E_2), (3*R*)-1,7-diphenyl-(4*E*,6*E*)-4,6-heptadien-3-ol (DPHD; a phytoestrogen from *C. comosa*), powdered *C. comosa* rhizomes or its crude ethanol extract. They were then analyzed by liquid chromatography–mass spectrometry, characterized, and subjected to the orthogonal projections to latent structures discriminant analysis statistical model to identify tentative biomarkers.
Results: Levels of five classes of lipids (ceramide, ceramide-1-phosphate, sphingomyelin, 1-*O*-alkenyl-lysophosphatidylethanolamine and lysophosphatidylethanolamine) were elevated in the OVX rats compared to those in the SHAM rats, while the monoacylglycerols and triacylglycerols were decreased. The E_2 treatment only reversed the levels of ceramides, whereas treatments with DPHD, *C. comosa* extract or powder returned the levels of all upregulated lipids back to those in the SHAM control rats.
Conclusions: The findings suggest the potential beneficial effects of *C. comosa* on preventing the increased ceramide levels in OVX rats, a possible cause of metabolic disturbance under estrogen deficiency. Overall, the results demonstrated the power of untargeted lipidomics in discovering disease-relevant biomarkers, as well as evaluating the effectiveness of treatment by *C. comosa* components (DPHD, extract or powder) as utilized in Thai traditional medicine, and also providing scientific support for its folklore use.

1. Introduction

Lipidomics is a subset of metabolomics, which is used for quantitatively measuring and comparing levels of lipidomes—the complete collection of lipids in a living system—in response to stimuli of interest, such as diseases (Wenk, 2005). Defined by their solubility in organic solvents, the different classes of lipids can be structurally distinct, and many have been reported to relate to the

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cause of diseases (Gross and Han, 2006; Hu et al., 2009). For example, monoalkylglycerol ether (MAGE) was found to promote both invasiveness in cancer cells (Chiang et al., 2006) and adipocyte differentiation, which is etiologically linked to obesity and diabetes (Homan et al., 2011). Because lipidomics provides a comprehensive view of the overall state of living systems, it is potentially a powerful tool for evaluation of the therapeutic effectiveness of treatment by medicines.

Estrogen plays many important regulatory functions in physiological processes in females, such as the development and regulation of reproductive tissue functions, management of energy, maintenance of body weight and functioning of bones. The natural decline in the estrogen level during menopause contributes to the increased risk of several diseases, including obesity, cardiovascular diseases and osteoporosis (Nilsson and Gustafsson, 2002). While the administration of estrogen to women can lower the risk as well as prevent or alleviate these symptoms, the possible carcinogenic effects of estrogen administration have limited its long-term usage (Beral et al., 2002; Lethaby et al., 2004). Many studies are, therefore, focused on searching for safer alternatives for post-menopausal treatment, and in particular for phytoestrogens.

Curcuma comosa Roxb. (Zingiberaceae family) has been used in Thai traditional medicine for the treatment of postpartum uterine bleeding, gynecological problems and inflammation (Anonymous, 1967; Pongboonrod, 1976; Weerachayaphorn et al., 2010, 2011). The major active compound in *C. comosa* rhizomes is the diarylheptanoid (3*R*)-1,7-diphenyl-(4*E*,6*E*)-4,6-heptadien-3-ol (DPHD) (Suksamrarn et al., 2008), which was reported to exhibit estrogenic-like activities at the transcriptional level similar to those of estrogen (17β-estradiol; E_2) (Winuthayanon et al., 2009a, 2009b). In addition, the crude *C. comosa* rhizome extract from hexane and DPHD were reported to lower plasma lipids (Piyachaturawat et al., 1999; Prasannarong et al., 2012), to enhance the differentiation and function of both mouse (Bhukhai et al., 2012) and human (Tantikanlayaporn et al., 2013a) osteoblastic cells via an estrogen receptor pathway *in vitro*, and to prevent bone loss in estrogen-deficient ovariectomized (OVX) rats after 12 weeks of treatment *in vivo* (Tantikanlayaporn et al., 2013b). However, while these pharmacological properties rendered DPHD a promising candidate for the treatment of estrogen-deficient postmenopausal osteoporosis in women (Tantikanlayaporn et al., 2013b), its effects at the metabolic level and on the metabolic pathways, which may suggest the overall state of the organism, have not been examined.

To understand the metabolic changes that result from estrogen deficiency, previous works have applied metabolomics analysis to study the serum (Ma et al., 2011) and plasma (Ma et al., 2013) of OVX rats compared to that of sham-operated controls. Using gas chromatography coupled to mass spectrometry (MS), elevated levels of some unsaturated fatty acids (octadecadienoic and arachidonic acids), amino acids (glutamic acid, leucine, isoleucine and valine), cholesterol, homocysteine, 3-hydroxybutanoic acid, glucose and glycerol were reported (Ma et al., 2011, 2013). On the other hand, the levels of some other glycolytic intermediates (alanine and glyceraldehyde 3-phosphate), tricarboxylic acid cycle-associated metabolites (citric acid and succinic acid), sugars (galactopyranose and arabinofuranose) and an unsaturated fatty acid (docosahexaenoic acid) were decreased in the OVX rats. While these studies comprehensively linked these metabolites to many important metabolic pathways, knowledge about the involvement of lipids in OVX rats is still limited. Because lipids are involved in energy storage, cellular structures, signal transduction and the regulation of physiological processes, as well as in the formation of many chronic diseases (Vinayavekhin et al., 2010), and because *C. comosa* has been demonstrated to lower lipid levels in OVX animals (Prasannarong et al., 2012), characterization of the lipid profile may provide a better understanding of the responses.

The present study applied a liquid chromatography (LC)–MS-based untargeted lipidomics approach to study the lipid changes in OVX rat serum with and without treatment with *C. comosa*. First, the changes in serum lipids associated with OVX rats were characterized to identify potentially novel biomarkers and link them to metabolic pathways. Subsequently, the levels of these specific modulated lipids were used to evaluate the effectiveness of the treatment of OVX rats with E_2, DPHD and *C. comosa* as either a crude ethanol extract or powder.

2. Materials and methods

2.1. Chemicals and plant materials

The preparation of standard *C. comosa* extract and isolation of DPHD were conducted as previously described (Suksamrarn et al., 2008; Tantikanlayaporn et al., 2013a). Briefly, *C. comosa* rhizomes were purchased from Kampaengsaen district, Nakornpathom, Thailand. A voucher herbarium specimen has been deposited at the Department of Plant Science, Faculty of Science, Mahidol University, Bangkok (SCMU No. 300). They were sliced into small pieces, dried and ground. The *C. comosa* extract was obtained by extracting *C. comosa* three times with 3 volumes of boiled ethanol (95%) at boiling point and removing the pooled solvent *in vacuo* to leave the dark brown viscous liquid. To obtain *C. comosa* fine powder, the dried ground *C. comosa* rhizomes were simply passed through a sieve no. 80. The composition of the *C. comosa* extract and *C. comosa* powder were evaluated by high performance liquid chromatography (HPLC)–ultraviolet (UV; 302 nm) fingerprints of the major diarylheptanoids in the samples and compared with those in our records (Supplementary Information (SI), Fig. S1). The *C. comosa* extract and *C. comosa* powder contained 87.5 and 21.0 mg DPHD per gram, respectively. The E_2 was purchased from Sigma-Aldrich Chemical Co. (MO, USA) and was employed as a positive control.

2.2. Animal treatment and sample collection

All animal protocols were approved by the committee on Animal Care and Use, Faculty of Science, Mahidol University (protocol no. MUSC 56-031-293). Sprague-Dawley female rats (8 weeks old; body weight 200–220 g) were supplied by the National Laboratory Animal Center of Thailand (Salaya, Nakornpathom, Thailand). They were housed in standard stainless steel cages in rooms at a controlled temperature (approximately 25 ± 2 °C) and relative humidity (50–60%) under 12-h light/dark cycle with free access to food (rat pellets, C.P. rat feed, Pokphand Animal Fed Co. Ltd., Bangkok, Thailand) and water *ad libitum*. The rats were subjected to similar surgical procedures as described previously (Tantikanlayaporn et al., 2013b). They were randomly assigned to either (i) a sham operation (SHAM, n=6) or bilateral ovariectomy (OVX, n=36). The OVX rats were then randomly divided into one of the following six groups (n=6 each) that received: (ii) no further treatment (OVX-control); (iii) 10 μg E_2/kg body weight (Bw), administered by subcutaneous (s.c.) injection (OVX+E_2); (iv) 50 mg DPHD/kg Bw, s.c. (OVX+DPHD); (v) 500 mg *C. comosa* extract/kg Bw, intragastric (i.g.) (OVX+E500); (vi) 1000 mg *C. comosa* powder/kg Bw, i.g. (OVX+P1000) and (vii) 2000 mg *C. comosa* powder/kg Bw, i.g. (OVX+P2000). The E_2 and DPHD were initially dissolved in absolute ethanol and then diluted in olive oil to give a final injection volume of approximately 100–200 μl. The extract and powder were directly suspended in 1% carboxymethyl cellulose to the specified concentration for oral ingestion of not more than 1 ml. All treatments were given daily for 12 weeks as