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Research Paper

Lipidomic profiling reveals significant alterations in lipid biochemistry in hypothyroid rat cerebellum and the therapeutic effects of *Sini* decoction

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ABSTRACT

Hypothyroidism is known to be closely associated with lipid metabolism. Although our previous serum and urine metabonomics studies have provided some clues about the molecular mechanism of hypothyroidism at the metabolic level, the precise mechanism underlying the pathogenesis of hypothyroidism remains elusive, especially from the aspect of lipid metabolism. In the present study, we applied an ultra high performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOF-MS)-based lipidomics method to analyze the global lipid profiles of hypothyroidism in rat cerebellum. Using unsupervised analysis and multivariate statistical analysis, we separated the Sham and hypothyroid groups clearly and screened out 23 potential lipid biomarkers related to hypothyroidism that were primarily involved in sphingolipid metabolism, glycerophospholipid metabolism and β-oxidation of fatty acid. Subsequently, we conducted computational analysis to build and simulate the lipid network of hypothyroidism, knowing that it would be useful to elucidate the pathological mechanism of hypothyroidism. Based on the selected 23 lipid biomarkers, we systematically evaluated the therapeutic effects of *Sini* decoction (*SND*) and the positive drug T_4. The results showed that both *SND* and T_4 can to some extent convert the pathological status of hypothyroidism through different pathways. Overall, this investigation illustrates that lipidomic profiling approach is powerful in giving a complementary view to the pathophysiology of hypothyroidism and offers a valuable tool for systematic study of the therapeutic effects of *SND* on hypothyroidism at lipid level.

1. Introduction

Hypothyroidism is a common endocrine disorder resulting from deficiency of thyroid hormone (TH), which is usually accompanied with motor-skill deficiencies and retarded intellectual development (Anderson, 2008). TH has long been recognized to play a critical role in mammalian brain development, especially in the cerebellum (Chatonnet et al., 2011; Koibuchi et al., 2003). Significant progress has been made in understanding the molecular action of TH in the cerebellum, manifested by the regulation of specific genes, transcription factors and neurotrophins (Anderson et al., 1998; Chantoux and Francon, 2002; Koibuchi and Chin, 1998; Neveu and Arenas, 1996). Therefore, the cerebellum is one of the most important brain organs targeted by TH. It is also known that lipid profiles are greatly altered in hypothyroidism and other thyroid disorders (Duntas and Brenta, 2012). Extensive research has been conducted to identify and annotate the key genes and enzymes involved in the effects that TH has on lipid metabolism in hypothyroidism (Choi and Choi, 2000; Johansson et al., 2005; Shin and Osborne, 2003). However, it is especially unfortunate that the study of monitoring integral alterations of lipid metabolites related to hypothyroidism in TH targeted organ, namely the cerebellum, particularly in a systemic and systematic way, is relatively insufficient.

Lipidomics, the systems-level analysis and characterization of lipids and their interacting moieties in an organism (Brown, 2012; Wenk, 2005), is an emerging field of biomedical research. As an important component of metabonomics, lipidomics opens a window to investigate molecular mechanisms related to cellular phenotype and discover lipid biomarkers in certain diseases (Patti et al., 2012).

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Rapid advances in lipidomics, particularly in mass spectrometry and computational methods have facilitated a comprehensive understanding of cellular lipid networks in living organisms. Last decades witnessed the development of novel methods to achieve maximum coverage of various species of lipids based on different analytical platforms, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography–mass spectrometry (GC–MS) (Heilmann and Heilmann, 2013; Holland et al., 2003; Quehenberger et al., 2011). Each approach can produce complementary findings on the same samples. GC–MS, which takes advantage of its library searching, can provide structure information and confident identification for unknown metabolites (Garcia and Barbas, 2011). Even so, LC–MS-based lipidomics has been gaining growing popularity in global lipidome investigation. An important reason is that some lipid molecules bearing formal charges, such as phospholipids, cannot be analyzed directly by GC–MS, but LC–MS can offer an alternative, and often much faster, solution (Patterson et al., 2011). For quantification analysis, bias may occur during the hydrolysis and derivatization processes required in GC–MS analysis. Therefore, LC—MS approach has been widely applied in lipidomics research in recent years (Ji et al., 2012; Jove et al., 2013; Zhou et al., 2012).

Up to now, there is only one report successfully delineating the global changes in cerebellar metabolic physiology related to hypothyroidism by employing GC–MS based metabolomics (Constantinou et al., 2011). However, the very nature of GC instantly discriminates against involatile and thermally labile compounds, which may lead to the loss of some important metabolite information, especially lipid information. For this reason, our previous studies (Wu et al., 2013a; Wu et al., 2013b) used an UHPLC/TOF-MS based metabonomics method to comprehensively characterize the metabolic alterations of hypothyroidism in rat urine and serum in three typical hypothyroid rat models. In particular, we found that 70% of the serum biomarkers and some biomarkers in rat urine were related to lipid metabolism. Although these encouraging results have enhanced our understanding of the pathobiological mechanism of hypothyroidism and offered some ideas about the close relationship between hypothyroidism and lipid metabolism, the precise mechanism underlying the effects of TH on lipids remains obscure.

Sini Decoction (*SND*), a classical formula in Traditional Chinese Medicine (TCM), has long been used to treat hypothyroidism in China and other Asian countries and regions (Miaorong and Xiaoying, 2010; PR China, 2010). It is composed of three medicinal herbs: *Acontium carmicharli*, *Glycyrrhiza uralensis* and *Zingiber officinale*. Our previous research also indicated that *SND* can exert recovery effects on hypothyroidism through regulating multiple metabolic pathways (Wu et al., 2013a; Wu et al., 2013b). Nevertheless, whether *SND* plays its therapeutic role on hypothyroidism by reversing disturbed lipid metabolism is still an open question; if it proves to be true, what is the underlying molecular mechanisms responsible for this therapeutic effect?

To shed light upon these questions, an UHPLC/TOF-MS-based lipidomics approach was applied to characterize the integral lipid alterations in the cerebellum of hypothyroid rat, and also investigate the therapeutic effects of *SND* on hypothyroidism at the lipid level. Here, we follow up on our previous studies and focus specifically on lipids and the cerebellum, rather than on the entire metabolome and organism. To the best of our knowledge, this is the first report of using the LC–MS-based lipidomics analysis to profile holistic lipid changes in the rat cerebellum related to hypothyroidism and its application in TCM. We found several altered lipid families, then complemented our lipidomics approach with a computational network analysis to identify detailed lipid pathways that may involve these altered lipids and the associated enzymes and genes.

2. Materials and methods

2.1. Ethics statement

All animal experiments were approved by the Administrative Committee of Experimental Animal Care and Use of the Second Military Medical University (SMMU, License no. 2011023), and conformed to the National Institute of Health guidelines on the ethical use of animals.

2.2. Reagents and materials

Reagents and materials used in this study were triiodothyronine (T_3) radioimmunoassay kit, thyroxin (T_4) radioimmunoassay kit and thyroid stimulating hormone (TSH) radioimmunoassay kit (Beijing North Institute of Biological Technology, Beijing, China); HPLC-grade methanol and acetonitrile (ACN) (Merck, Darmstadt, Germany); formic acid (Fluka, Buchs, Switzerland); ammonium formate, ketamine hydrochloride, xylazine, acepromazine, ketorolac, gentamicin and standard arachidonic acid (Sigma-Aldrich, St Louis, MO, USA); sphinganine and phytosphingosine (Acros Organics, NewJersey, USA); sphingosine, PC species including PC (15:1/0:0) and LysoPC (16:0) (Avanti Polar Lipids, inc, USA); leucic acid, palmitic acid and linoleic acid (Shanghai Jingchun Reagent Co); ultrapure water prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA); and chloroform and other reagents were of analytical grade.

Acontium carmichaeli (from Sichuan, China), *Glycyrrhiza uralensis* (from Xinjiang, China) and *Zingiber officinale* (from Guizhou, China) were purchased from Lei Yunshang Medicine Corp. (Shanghai, China). They were authenticated by Lianna Sun (Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, China) and met the standards recorded in Chinese Pharmacopoeia (2010 Edition).

2.3. Preparation of SND samples

According to the original composition of *SND* recorded in Chinese Pharmacopoeia 2010 Edition, *SND* was prepared using the following procedures. The crude drugs of *Aconitum carmichaeli* 90 g, *Zingiberofficinale* 60 g and *Glycyrrhiza uralensis* 90 g were immersed in 2.4 l water for 1 h and then decocted to boil for 2 h. The decoction was filtered through four layers of gauze. Next, the drugs were boiled once again for 1 h with 1.9 l of water and the decoction was filtrated out using the above method. Afterward, the successive decoctions were merged and condensed under reducing pressure using the rotary evaporator. Finally, the extraction solution was made to a concentration of 1.0 g crude drugs/ml. According to our previous published paper (Tan et al., 2011), 53 components of *SND* were identified. To ensure the reproducibility of *SND* extracts, *SND* extraction procedure was conducted five times parallell. The results showed that relative standard deviation (RSD) of the peak area of 10 typical compounds in *SND* was less than 3%, suggesting that the *SND* samples were qualified for further experience.

2.4. Animal experiments

The study was approved by the national legislation of China and local guidelines and performed at the Center of Laboratory Animals of the Second Military Medical University (Shanghai, China). Forty male Wistar rats (175 ± 10 g) commercially obtained from the Slac Laboratory Animal Co., LTD (Shanghai, China) were maintained under standard laboratory conditions (temperature of 21–23 °C, relative humidity 45–65%, and 12 h/12 h light/dark cycle) with aseptic food and tap water ad libitum. After one-week habituation, all animals were housed individually in metabolism cages and allowed to