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Identification of berbamine dihydrochloride from barberry as an anti-adipogenic agent by high-content imaging assay

Shifeng Wang [a], Qiao Zhang [a], Yuxin Zhang [a], Yanling Zhang [a], Qinghua Wu [b], Shiyou Li [c,*], Yanjiang Qiao [a,**]

[a] School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, China
[b] HD Biosciences Co., Ltd., Shanghai 201201, China
[c] Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, China

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Abstract *Objective:* Lipid droplet (LD) deposition in adipose tissue is a critical factor leading to metabolic dysfunction. Various herbal medicines in traditional Chinese medicine (TCM) are used to treat hyperlipidemia, type 2 diabetes, obesity, and other diseases. The objective of this study was to identify potential anti-adipogenic agents from TCM herbal compounds.
Methods: One hundred and twenty compounds were evaluated in terms of their effect on adipocyte differentiation through image-based high content screening. Anti-adipogenic effects of identified hits were further confirmed at various concentrations. In addition, drug-induced liver injury assay was performed with HepG2 cells to test the hepatotoxicity of hit compounds.
Results: Berbamine (BBM), a chemical isolated from barberry, and a derivative of BBM, berbamine dihydrochloride (BBMD), reduced LDs formation by more than 50%. Dose-dependent effects were observed and the IC_{50} values of the two hits, BBM and BBMD, were determined as 1.88 μM and 0.95 μM, respectively. Moreover, BBM induced mild HepG2 cell injury, while its dihydrochloride—BBMD did not exhibit hepatotoxicity within 40 μM.
Conclusion: This study demonstrates that BBMD may be a potential therapeutic candidate for disorders associated with elevated LDs accumulation.

* Corresponding author. Fax: +86 10 8049 7628.
** Corresponding author. Fax: +86 10 8473 8661.
E-mail addresses: lishiyou@big.ac.cn (S. Li), yjqiao@bucm.edu.cn (Y. Qiao).
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Introduction

Lipid droplets (LDs) are dynamic intracellular organelles that play an important role in cellular lipid storage and trafficking.[1] LDs mainly contain triglycerides, the metabolic precursor diacylglycerol,[2] and perilipin.[3] Overloaded intracellular LDs are associated with metabolic diseases such as obesity, insulin resistance, non-alcoholic fatty liver disease (NAFLD), and type 2 diabetes.[4] Recent research by Tirinato et al[5] showed that high levels of LDs were present in colorectal cancer stem cells, and may thus be a cellular target for innovative anticancer therapies. LDs are mainly stored in adipose tissue. Also, 3T3-L1 preadipocyte derived from Swiss mouse embryo tissue is a typical fat-storage cell line that differentiates into mature adipocytes when stimulated by hormone cocktail, consisted by insulin, dexamethasone, and 3-Isobutyl-1-methylxanthine (IBMX).[6] In research, 3T3-L1 adipocytes were used as a target for the discovery of anti-adipogenic drugs.[7]

Currently, there are three main approaches for quantifying LDs. The first (conventional) method, which is labor intensive and time consuming, is to use oil red O staining and signal acquisition by ultraviolet spectroscopy. The second method is a label-free method, with bright-field microscopy[8] and spectroscopy imaging[5] as examples. The third method applies lipophilic fluorescent dyes specific for neutral LDs,[9] which makes high-content detection of LDs in 3T3-L1 adipocytes feasible. These assays have facilitated discovery of novel anti-adipogenic ingredients.

Metabolic disorders generally require long-term intake of lipid-regulating drugs. The liver is the primary organ for both fat and drug metabolism, and is thus subjected to potential hazardous agents. Therefore, during drug development, attention must be paid to drug safety. For example, individuals with NAFLD are exposed to a high risk of drug-induced liver injury (DILI) during long-term consumption of anti-hyperlipidemia drugs.[10] Using drug-induced liver toxicity assays, with high sensitivity and specificity developed by high throughput format[11–13] is paramount for predicting the safety of the identified hits at early stage of drug discovery.

In China, many traditional herbs are prescribed for regulating lipid metabolism, such as milk thistle (*Silybum marianum* (L.) Gaertn.),[14] salvia root (*Salvia miltiorrhiza* Bunge), astragalus root (*Astragalus membranaceus* (Fisch.) Bunge),[15] cassia seed (*Senna obtusifolia* (L.) H.S.Irwin & Barneby),[16] and coptis rhizome (*Coptis chinensis* Franch.).[17] However, understanding of the molecular bioactivities of these plants remains incomplete. The aim of the present study was to screen and identify novel anti-adipogenic agents from Chinese herbs and to predict their hepatotoxicity using a high-content imaging assay.

Materials and methods

Herbal compounds and reagents

A total of 120 traditional Chinese medicine (TCM) herbal compounds with diverse chemical structures were purchased from the Beijing Institute for Drug Control (Beijing, China) and Dalian Institute of Chemical Physics (Dalian, China). The purity of all the compounds was >98%. The test compounds were derived from Chinese herbs that are commonly prescribed and are thus readily available.

Compounds were dissolved in dimethyl sulphoxide (DMSO) and immediately stored at −20°C as a stock solution for testing. A ToxInsight™ Drug Induced Liver Injury (DILI) Cartridge was purchased from Thermo Scientific (Waltham, MA, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco (Grand Island, NY, USA). Dexamethasone, 3-isobutylxanthine (IBMX), lovastatin, Nile Red, Hoechst 33342, and all other chemicals were purchased from Sigma–Aldrich (Saint Louis, MO, USA), if not otherwise stated.

Induction of adipogenic differentiation

3T3-L1 pre-adipocytes (American Type Culture Collection, Manassas, VA, USA) were cultured in 10-cm dishes in DMEM supplemented with 10% FBS, 100 unit/mL penicillin, and 100 μg/mL streptomycin. Cultures were maintained in a humidified atmosphere of 5% CO_2 in air at 37°C. 3T3-L1 adipocytes differentiation was performed as reported previously.[18] Plating of cells was defined as Day 1. Initiation of differentiation was conducted on Day 4, and promotion of differentiation was done on Day 6 using insulin medium (1 μg/mL). The differentiation medium comprised 0.5 mM IBMX, 1 μg/mL insulin, 0.25 μM dexamethasone, and 2 μM rosiglitazone. Adipocytes were incubated further in DMEM complete culture medium for 2 days. For determination of the Z′ factor, 30 differentiated and 30 undifferentiated wells were performed in parallel.

Chemical treatment

TCM test compounds were added to confluent 3T3-L1 pre-adipocytes from Day 3, and were continuously present for 6 days. During primary screening, 120 test compounds were prepared at a concentration of 4 mM in 100% DMSO, and then diluted into 100 μM using DMEM complete culture medium. For dose–response titration, tested compounds were three-fold serially diluted in DMSO from 12 mM stock solutions and were further diluted into complete culture medium. Three replicates were performed in parallel for each dose.

Staining and imaging of 3T3-L1 adipocytes

By the end of cell differentiation (Day 10), culture media were removed and cells were rinsed gently with phosphate-buffered saline (PBS). First, 100 μL of 4% paraformaldehyde solution was added to each well and the plates maintained at 25°C for 20 min, followed by rinsed with PBS. Next, 100 μL of 5 μg/mL freshly prepared Nile Red solution was applied to each well and incubated at 25°C for 10 min. Then Nile Red solution was removed and the plate was rinsed with PBS. Finally, 10 μg/mL Hoechst 33342 was added to cells and kept at 25°C for 15 min, followed by rinsed with PBS.

Signals of lipid droplets and nuclei staining in 3T3-L1 adipocytes were captured using the imaging platform Cellomics ArrayScan® VTI HCS Reader (Thermo Scientific) with a 10 × objective lens; eight fields per well were scanned.