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Using molecular docking screening for identifying hyperoside as an inhibitor of fatty acid binding protein 4 from a natural product database

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ABSTRACT

The inhibition of fatty acid binding protein 4 (FABP4) by using small molecules could potentially provide therapeutic opportunities for metabolic disorders treatment. According to the results of our in-house virtual screening on the herbal molecules database, this study reports flavonols as an ideal scaffold for FABP4 inhibitors development. Among the popular flavonols examined, we identified hyperoside as a promising FABP4 inhibitor. Identical to the well-known FABP4 inhibitor BMS309403, hyperoside induced lipid accumulation and upregulated peroxisome proliferator-activated receptor γ (PPARγ) protein expression during the adipocyte differentiation process. Furthermore, both PPARγ antagonist and FABP4 overexpression attenuated hyperoside-induced adipogenesis, indicating that hyperoside promoted adipogenesis in adipocytes via the FABP4/PPARγ pathway. We anticipate hyperoside to be a promising, novel FABP4 inhibitor for antidiabetic drug development.

1. Introduction

Fatty acids are a class of straight-chain hydrocarbons with a carboxyl tail group. They are metabolized to yield large quantities of adenosine triphosphate (ATP), thus acting as a preferred fuel for the human metabolism. Abnormally elevated fatty acid levels in circulation have been reported to be involved in the pathogenesis of chronic metabolic disorders, including type 2 diabetes, obesity, and atherosclerosis (reviewed in Currie, Schulze, Zechner, Walther, & Farese, 2013; Ford, 2010; Konige, Wang, & Sztalryd, 2014). Fatty acid trafficking in cells requires fatty acid binding proteins (FABPs), a family of approximately 15 kDa lipid chaperones. Both endogenous and exogenous fatty acids can reversibly bind to FABPs with high affinity, and the fatty acid-bound FABPs are shuttled in the

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cytoplasm for the lipid metabolic process or storage (review in Boord, Fazio, & Linton, 2002; Furuhashi & Hotamisligil, 2008).

The adipocyte FABP, also known as FABP4, is predominantly expressed in mature adipocytes and adipose tissue. FABP4, as a critical carrier of fatty acids, plays an important role in the pathogenesis of various metabolic disorders, including insulin resistance, diabetes, and atherosclerosis. Mice fed with a high-fat-diet exhibit a representative insulin resistance phenotype; however, this insulin resistance is absent in FABP4-deficient mice during obesity progression. Furthermore, FABP4-deficient mice on a high-fat diet performed better in both insulin and glucose tolerance tests. Furuhashi et al. reported that, in addition to these genetic approaches, the inhibition of FABP4 by pharmacological interventions could potentially mimic the phenotype of FABP4-deficient mice (Furuhashi et al., 2007). Therefore, pharmacological agents that inhibit FABP4 activity could provide therapeutic opportunities for treating these metabolic disorders, such as insulin resistance, diabetes, and atherosclerosis (Lehmann et al., 2004).

To date, several small molecules have been identified as novel FABP4 inhibitors, but only BMS309403 has been systematically studied in both *in vitro* and *in vivo* diabetic models (Furuhashi et al., 2007). Although the antidiabetic effects of BMS309403 have been well validated in mouse models, it has yet to be approved for clinical use. Expenditures on clinical trials have considerably increased over the past two decades, which has driven pharmaceutical companies to adopt flexible strategies in order to reduce the cost of new drug development. One attractive approach is to identify new indications for existing drugs, which is known as drug repositioning or drug repurposing (Bisson, 2012; Oprea & Mestres, 2012). In our previous repurposing study, 10 U.S. Food and Drug Administration (FDA)-approved drugs were identified as novel FABP4 inhibitors using molecular docking screening. Moreover, the inhibitory effects on the FABP4 activity of levofloxacin, trovafloxacin and pimozide have been validated using ligand displacement assay and cell-based function assays. Therefore, we suggest that these 3 drugs could be explored further as potential candidates for the treatment of metabolic diseases (Wang et al., 2014a, 2014b).

Apart from synthetic compound libraries, phytochemicals naturally occurring in herbs are an alternative source of chemical candidates for identifying potential agents for metabolic disease treatment. Many popular herbs have been widely used as alternative medicines for thousands of years. According to modern pharmacological experiments, evidence supporting the benefits of these herbs is satisfactorily convincing. In addition, the chemical structures of major bioactive components extracted from these herbs have been characterized adequately. Therefore, identifying the lead compounds from these phytochemicals is another promising strategy for reducing the cost of new drug development (review in Newman & Cragg, 2007, 2012). In this study, we identified well-known phytochemicals from our in-house natural product database, specifically novel FABP4 inhibitors, by conducting molecular docking analysis (Wang et al., 2013).

Peroxisome proliferator-activated receptor gamma (PPARγ), a PPARG-encoded nuclear receptor, is a key gene regulator for fatty acid storage and glucose metabolism. The activation of PPARγ promotes the differentiation of adipocytes and enhances insulin sensitivity (Ahmadian et al., 2013). Thiazolidinediones (TZD), a widely used antidiabetes medication, directly activates PPARγ and enhances insulin sensitivity (Saltiel & Olefsky, 1996). FABP4 has recently been reported to interact directly with PPARγ (Adida & Spener, 2006),triggering ubiquitination and subsequent proteasomal degradation of PPARγ (Garin-Shkolnik, Rudich, Hotamisligil, & Rubinstein, 2014). A well-known FABP4 inhibitor, BMS309403, elevates the basal protein levels of PPARγ, therefore stimulating adipogenesis in adipocytes. The fatty acid-binding pocket of FABP4 is distinct from the PPARγ interaction site (Garin-Shkolnik et al., 2014). Therefore, BMS309403 binding to FABP4 might lead to a conformational change in FABP4, resulting in elevated PPARγ protein expression. The PPARγ functions as a key regulator in insulin sensitivity; therefore we hypothesized that an ideal FABP4 inhibitor for antidiabetic drug development should not only inhibit FABP4, but also upregulate PPARγ protein expression and induce adipogenesis in adipocytes. We also tested the potential effects of the newly identified FABP4 inhibitors from phytochemicals on PPARγ protein expression and adipogenesis in adipocytes.

2. Methods

2.1. *Molecular docking screening*

The natural product database was established as previously described (Wang et al., 2013). Molecular docking screening was performed using the three-dimensional crystal structures of substrate-free fatty acid binding protein 4 (FABP4) obtained from the Protein Data Bank (PDB, http://www.rcsb.org/pdb/home/home.do). Of 15 human FABP4 crystal models provided in the PDB (accessed on November 30, 2012); we selected 4 protein models for our docking screening, including PDB code 1TOU (Ringom et al., 2004), 1TOW (Lehmann et al., 2004), 2NNQ (Sulsky et al., 2007) and 3FR5 (Barf et al., 2009). AutoDock Vina (version 1.0.2), a molecular docking screening program (downloadable at http://vina.scripps.edu/) developed in the Molecular Graphics Lab at The Scripps Research Institute (Trott & Olson, 2010), was used for all docking of the experiments in this study. The default values of the docking parameters in AutoDock Vina were maintained. A grid box of 20 Å × 14 Å × 12 Å encompassed the inhibitor binding cavity of FABP4. The binding modes were clustered through the root mean square deviation among the Cartesian coordinates of the ligand atoms. The docking results were ranked based on the binding free energy. The binding modes with the lowest binding free energy and the most cluster members were selected for optimum docking conformation. PyMOL (downloadable at https://www.pymol.org/) is an open-source visualization system that produces high-quality 3D images of small molecules and biological macromolecules (Schrödinger LLC, New York, NY, USA). The binding results in our study were illustrated using PyMOL Molecular Graphics System (version 1.3).

2.2. *Human FABP4 expression and purification*

Human FABP4 cDNA was isolated from a human brain cDNA library by using PCR. The amplified gene product was digested with KpnI and HindIII (New England Biolabs, Beverly,