Toxicology and Applied Pharmacology 273 (2013) 435–441

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

High Impact Short Article

Isoniazid suppresses antioxidant response element activities and impairs adipogenesis in mouse and human preadipocytes

Yanyan Chen [a,b], Peng Xue [a,d], Yongyong Hou [a,c], Hao Zhang [d], Hongzhi Zheng [a,b], Tong Zhou [a], Weidong Qu [d], Weiping Teng [b,*], Qiang Zhang [a], Melvin E. Andersen [a], Jingbo Pi [a,c,**]

[a] Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, 6 Davis Drive, Research Triangle Park, NC 27709, USA
[b] The First Affiliated Hospital, China Medical University, Shenyang 110001, China
[c] School of Public Health, China Medical University, Shenyang 110001, China
[d] Key Laboratory of the Public Health Safety, Ministry of Education, School of Public Health, Fudan University, Shanghai, China

ARTICLE INFO

Article history:
Received 26 July 2013
Revised 26 September 2013
Accepted 3 October 2013
Available online 12 October 2013

Keywords:
Isoniazid
Antioxidant response element
Nrf2
Preadipocyte
Adipogenesis
Adipocyte

ABSTRACT

Transcriptional signaling through the antioxidant response element (ARE), orchestrated by the Nuclear factor E2-related factor 2 (Nrf2), is a major cellular defense mechanism against oxidative or electrophilic stress. Here, we reported that isoniazid (INH), a widely used antitubercular drug, displays a substantial inhibitory property against ARE activities in diverse mouse and human cells. In 3T3-L1 preadipocytes, INH concentration-dependently suppressed the ARE-luciferase reporter activity and mRNA expression of various ARE-dependent antioxidant genes under basal and oxidative stressed conditions. In keeping with our previous findings that Nrf2-ARE plays a critical role in adipogenesis by regulating expression of CCAAT/enhancer-binding protein β (C/EBPβ) and peroxisome proliferator-activated receptor γ (PPARγ), suppression of ARE signaling by INH hampered adipogenic differentiation of 3T3-L1 cells and human adipose-derived stem cells (ADSCs). Following adipogenesis induced by hormonal cocktails, INH-treated 3T3-L1 cells and ADSCs displayed significantly reduced levels of lipid accumulation and attenuated expression of C/EBPα and PPARγ. Time-course studies in 3T3-L1 cells revealed that inhibition of adipogenesis by INH occurred in the early stage of terminal adipogenic differentiation, where reduced expression of C/EBPβ and C/EBPδ was observed. To our knowledge, the present study is the first to demonstrate that INH suppresses ARE signaling and interrupts with the transcriptional network of adipogenesis, leading to impaired adipogenic differentiation. The inhibition of ARE signaling may be a potential underlying mechanism by which INH attenuates cellular antioxidant response contributing to various complications.

Introduction

Pivotal to lipid homeostasis, energy balance and production of adipokines/cytokines, white adipose tissue (WAT) is a critical mediator of obesity-induced insulin resistance. Conversely, defects in adipogenesis, which impair the ability of WAT to store triglycerides, can also lead to reduced insulin sensitivity (Xue et al., 2013). Thus, abnormalities in adipose formation and function are crucial in the development of metabolic disorders, including insulin resistance and Type 2 diabetes (T2D). Adipogenesis is a complex process in which mesenchymal stem cells (MSCs) are first converted to fibroblast-like preadipocytes and then to mature, spherical adipocytes with lipid accumulation (Farmer, 2006; Lefterova and Lazar, 2009; Rosen and MacDougald, 2006; Tontonoz and Spiegelman, 2008). Although the regulation of the commitment of MSCs to preadipocytes is not fully understood, it is clear that terminal adipogenesis (preadipocytes to adipocytes) is regulated by a complicated network of transcription factors, including CCAAT/enhancer-binding proteins (C/EBPs) and peroxisome proliferator-activated receptor γ (PPARγ), that coordinate the expression of hundreds of proteins responsible for establishing the mature fat-cell phenotype (Farmer, 2006; Lefterova and Lazar, 2009; Rosen and MacDougald, 2006; Tontonoz and Spiegelman, 2008).

Nuclear factor erythroid-derived factor 2-related factor 2 (Nrf2, also known as Nfe2l2) is a CNC-bZIP transcription factor that is well-established as a master regulator of the cellular adaptive response to oxidative stress (Maher and Yamamoto, 2010; Pi et al., 2010b). Our recent studies demonstrated that Nrf2 plays a critical role in adipogenesis by regulating expression of C/EBPβ and PPARγ via the antioxidant response elements (AREs) (Hou et al., 2012; Pi et al., 2010a). Activation of ARE activity by Nrf2 occurs at the very early stage upon adipogenic hormonal challenge, leading to transcription of C/EBPβ. Deficiency of *Nrf2* in preadipocytes and mouse embryonic fibroblasts resulted in impaired adipogenesis (Hou et al., 2012; Pi et al., 2010a). In agreement with these findings, global *Nrf2*-knockout (KO) mice displayed decreased fat mass and are resistant to high fat

* Corresponding author.
** Correspondence to: J. Pi, School of Public Health, China Medical University, 92 Beier Road, Heping District, Shenyang, 110001, P. R. China.
E-mail address: jingbopi@gmail.com (J. Pi).

0041-008X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.taap.2013.10.005

diet (HFD)-induced obesity (Pi et al., 2010a). *Ob/ob* mice with whole-body or adipocyte-specific ablation of *Nrf2* showed reduced body weight and WAT mass, but interestingly, develop insulin resistance and hyperglycemia (Xue et al., 2013). These findings demonstrate a novel role of Nrf2 beyond its canonical xenobiotic detoxification and antioxidant response, suggesting that Nrf2 is a key transcription factor that controls terminal adipogenesis, lipogenesis, insulin sensitivity and glucose homeostasis.

Because Nrf2 is a master regulator of cellular defense against oxidative/electrophilic stress, targeting the Nrf2-ARE pathway represents an attractive strategy to prevent and treat a variety of chronic diseases (Kundu and Surh, 2010; Ren et al., 2011; van Muiswinkel and Kuiperij, 2005; Zhan et al., 2012). Thus, it is an urgent need to discover agents that can specifically activate or inhibit the ARE signaling pathway. To identify novel compounds that specifically modulate Nrf2-ARE activity, we have performed a series of chemical screens using an ARE-luciferase reporter stably expressed in 3T3-L1 cells (Hou et al., 2012) and several human cell lines, including HepG2 and HaCaT cells (Zhao et al., 2011). In the present study we identified and characterized isoniazid (INH), the most widely used antitubercular drug (Saukkonen et al., 2006), as a novel chemical inhibitor of ARE activity. This finding suggests that the common complications of INH therapy, including hepatotoxicity, may be related to the suppression of ARE-mediated adaptive antioxidant response by INH. Moreover, we demonstrated that INH, by suppressing ARE activity, inhibits adipogenesis through interfering with the expression of C/EBPβ and C/EBPδ during the early stage of adipogenesis, suggesting that INH treatment may impair the development and function of adipose tissues.

Materials and methods

Reagents. Insulin solution (human, I9278), INH (13377), 3-isobutyl-1-methylxanthine (IBMX, I7018), dexamethasone (D1756), indomethacin (I7378), *tert*-butylhydroquinone (tBHQ, 19986), sodium arsenite (iAs^{3+}, 71287), and Oil-red O (ORO, 75087) were purchased from Sigma (St. Louis, MO). Rosiglitazone maleate was obtained from SmithKline Beecham Pharmaceuticals (London, UK). Culture media, calf serum (CS), fetal bovine serum (FBS) and supplements were obtained from Life Technologies (Grand Island, NY).

Cell culture and differentiation. 3T3-L1 preadipocytes were obtained from ATCC (Manassas, VA) and maintained in high-glucose DMEM with 100 unit/ml penicillin, 100 μg/ml streptomycin, and 10% CS. Human adipose tissue-derived stem cells (ADSCs) were obtained from Life Technologies and cultured in the ADSC growth media (Life Technologies) according to the manufacturer's recommendation. To induce differentiation, confluent 3T3-L1 cells and human ADSCs were treated by using the DMI and DMIRI protocols, respectively (Hou et al., 2013). With the DMI protocol, cells were differentiated by replacing CS growth medium with DMI differentiation medium containing 1 μM dexamethasone, 0.5 mM IBMX and 1 μg/ml insulin in DMEM with 10% FBS. After 48 h the medium was changed to DMEM with 10% FBS and 1 μg/ml insulin (insulin medium), followed by an additional 3-day culture in DMEM with 10% FBS and 1 μg/ml insulin (Fig. S1, upper panel); With the DMIRI protocol, cells were differentiated by replacing growth medium with DMIRI differentiation medium containing 1 μM dexamethasone, 0.5 mM IBMX, 1 μg/ml insulin, 1 μM rosiglitazone and 125 μM indomethacin in DMEM with 10% FBS. After 48 h, cells were maintained for an additional 3 days in the same medium without additives but 10% FBS (Fig. S1, lower panel). Except time-course study, INH exposure during adipogenesis in 3T3-L1 cells and human ADSCs was performed as detailed in Fig. S1. Differentiation of preadipocytes to mature adipocytes was confirmed by ORO staining of lipid vesicles as detailed previously (Hou et al., 2012; Pi et al., 2010a). The ORO staining was quantified by the Bio-Rad Quantity One one-dimensional analysis software (Bio-Rad Laboratories, Hercules, CA). All cells were maintained at 37 °C in a 5% CO_2 environment.

Reverse transcription quantitative real-time PCR (RT-qPCR). Total RNA was isolated with TRIzol (Invitrogen) and subsequently subjected to cleanup by using an RNase-Free DNase Set and RNeasy Mini kit (Qiagen, Valencia, CA). The resultant DNA-free RNA was diluted in RNase-free H_2O and quantified by Nanodrop (Thermo, Wilmington, DE) at 260 nm. RNA samples were stored at −80 °C until use. Quantitative real-time RT-PCR was performed as described previously (Xue et al., 2013; Yang et al., 2012). The primers (sequences are listed in online material, Table S1) were designed by using Primer Express 4 (Life Technologies) and synthesized by Bioneer, Inc. (Alameda, CA). Total RNA was reverse-transcribed with MuLV reverse transcriptase and Oligo d(T) primers (Life Technologies). A SensiFAST SYBR Hi-ROX kit (BIOLINE USA Inc., Taunton, MA) was used for qPCR. Real-time fluorescence detection was performed by using an ABI PRISM 7900 HT Fast Real-time PCR System (Life Technologies).

Western blot analysis. Collection of cell lysates and Western blotting were performed as detailed previously (Hou et al., 2012; Xue et al., 2013). Antibodies for Nrf2 (sc-13032; 1:500), C/EBPα (sc-61; 1:500), C/EBPβ (sc-7962; 1:500) and MafF/G/K (sc-22831; 1:500) were from Santa Cruz, Inc. (Santa Cruz, CA). Antibodies for C/EBPδ (#2318; 1:1000), PPARγ (#2435; 1:1000), phosphorylated cAMP response element-binding protein (p-CREB, #9198; 1:1000), c-JUN (#9165; 1:1000), c-FOS (#2250; 1:1000) and CHOP10 (#2895; 1:1000) were purchased from Cell Signaling Technology, Inc. (Danvers, MA). Antibody for β-ACTIN (A1978; 1:2000) was purchased from Sigma. The molecular weight (MW) of each protein shown on immunoblot was estimated based on the MagicMark™ XP Western Protein Standard (Invitrogen) on 4–12% or 12% Tris-Glycine Gel (Life Technologies). Quantification of the results was performed by Bio-Rad Quantity One one-dimensional analysis software (Bio-Rad Laboratories).

ARE-luciferase reporter assay. Cignal Lenti ARE reporter, which expresses a luciferase gene driven by multiple ARE (TCACAGTGACTCAGCAAAATT) repeats, was obtained from SABiosciences (Frederick, MD). Lentiviral transduction of 3T3-L1 cells was performed as described previously (Hou et al., 2012; Pi et al., 2010a). Cells were grown to ~90% confluence and sub-cultured in medium containing 1.0 μg/ml of puromycin. The luciferase activity was measured by Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol. The luciferase activity was normalized to protein content or cell viability which was determined by using a Non-Radioactive Cell-Proliferation Assay Kit (Promega).

Statistical analyses. All statistical analyses were performed by using GraphPad Prism 4 (GraphPad Software, San Diego, CA), with $p < 0.05$ taken as significant. For comparisons among multiple groups, one-way ANOVA with Tukey's multiple comparison test or two-way ANOVA with the Bonferroni post hoc testing was performed.

Results

Identification of INH as an inhibitor of ARE-dependent gene expression

To identify novel chemical modulators of ARE activity, we have performed a series of chemical screening using an ARE-luciferase reporter stably expressed in 3T3-L1 cells and multiple human cell lines, which have been confirmed responsive to activation of Nrf2 (Hou et al., 2012; Zhao et al., 2011). As shown in Figs. 1A and B, non-cytotoxic concentrations of INH exhibited a concentration-dependent inhibitory effect on ARE-luciferase activity in 3T3-L1 cells under basal (no stressor) and iAs^{3+}-treated conditions. The inhibitory effect was also observed in HepG2 cells and HaCaT cells stably expressing the