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

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A B S T R A C T

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/EBPβ 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/EBPα 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.

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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 adipogenesis 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; Letterova and Lazar, 2009; Rosen and MacDougald, 2006; Tontonoz and Spiegelman, 2006). 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; Letterova and Lazar, 2009; Rosen and MacDougald, 2006; Tontonoz and Spiegelman, 2006).

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...
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 Surih, 2010; Ren et al., 2011; van Muuswinkel 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 antibacterial drug (Saikkonen 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 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), eut-butylhydroquinone (tBHQ, 19986), sodium arsenite (iAs3+, 71228), and Oil-red O (ORO, 75087) were purchased from Sigma (St. Louis, MO). Rosiglitazone maleate was obtained from SmithKline (DMI and DMIRI protocols, respectively (Hou et al., 2013). With the DMI protocol, con...
The cells were treated with INH (10 mM) and/or tBHQ (50 mM) to examine the mRNA expression of ARE-dependent genes under basal and tBHQ-treated conditions. Induced adipogenesis, INH-treated ADSCs showed, in a concentration-dependent manner, reduced levels of lipid accumulation and attenuated expression of PPARγ1 and PPARγ2.

INH suppresses ARE activity and expression of C/EBPβ and C/EBPδ during early stage of adipogenesis

C/EBPβ and C/EBPδ play a role at the early stage of adipogenic differentiation by sensing adipogenic stimuli and initiating expression of PPARγ and C/EBPα (Rosen and MacDougald, 2006). Upon exposure to adipogenic signals, such as DMI or DMI cocktail, C/EBPβ and C/EBPδ are rapidly and transiently expressed, which transcriptionally regulate the expression of PPARγ and C/EBPα. Our previous studies have revealed that a transient activation of Nrf2 occurs upon initiation of differentiation by DMI or DMI cocktail, followed by an immediate induction of C/EBPβ and C/EBPδ. In keeping with the previous finding, here we found that INH significantly suppressed the elevated ARE activity in the early stage of DMI-induced adipogenesis (Fig. 4A). Importantly, this suppression is associated with a significant reduction in the mRNA and/or protein expression levels of C/EBPβ and in particular C/EBPδ (Figs. 4B–D), suggesting that INH may interfere with ARE activation in the early stage of adipogenesis and thus impair terminal differentiation.

Discussion

INH is the first-line medication to prevent and treat tuberculosis. INH hepatotoxicity is a common complication of antituberculosis therapy, although the molecular mechanisms are still unclear (Saukkonen et al., 2006). In the present study, we identified, for the first time, that INH is an effective inhibitor of ARE activity. Even at concentrations far less than causes tangible cytotoxicity, INH readily suppresses the expression of many ARE-dependent antioxidant and phase II detoxification genes, including Nqo1, Ho1, and Gclc, under both basal and oxidative stressed conditions in 3T3-L1 cells (Fig. 1) and human hepatocyte HepG2 cells (data not shown). Consistent with our findings, INH has been shown by others to diminish the levels of glutathione and the activity of glutathione peroxidase and catalase in the rat liver (Attri et al., 2001; Sodhi et al., 1996). Supplementation with antioxidant N-acetylcysteine protected animals against INH-induced hepatotoxic injury (Attri et al., 2001). Together, these findings including the present study suggest that the toxic effects of INH, including hepatotoxicity, may be due partly to oxidative stress resulting from inhibition of ARE-dependent antioxidant gene expression.

Our previous studies demonstrated that Nrf2 plays a critical role in adipogenesis by regulating the transcription of PPARγ via AREs (Pi et al., 2010a). In the present study, we found that INH, at concentrations suppressing ARE activity, dramatically delays and decreases hormonal cocktail DMI-induced PPARγ expression and adipogenesis in mouse preadipocytes and human ADSCs. Adipogenesis involves a sequential cascade of gene expression events coordinated by transcription factors that simultaneously induce tissue-specific gene expression and repress alternative cell fates. At the center of the network PPARγ is the principal regulator, which oversees the entire terminal differentiation process (Farmer, 2006; Rosen and MacDougald, 2006; Tontonoz and Spiegelman, 2008). The PPARγ gene is transcribed from alternative promoters that give rise to two major protein isoforms, PPARγ1 and γ2 (Zhu et al., 1995). Because the expression of both isoforms of PPARγ, but not C/EBPα, is partially dependent on Nrf2 (Hou et al., 2012; Pi et al., 2010a), INH-induced downregulation of PPARγ during adipogenesis may be attributed, at least in part, to the suppression of Nrf2-ARE activity. It has been well documented that PPARγ and C/EBPα form a positive feedback loop by activating each other’s expression and play roles at a later stage by inducing and maintaining expression of adipocyte-specific genes (Shao and Lazar, 1997; Wu et al., 1999). Thus, the reduction of C/EBPα during adipogenesis caused by INH treatment could be a secondary consequence of reduced expression and activity of PPARγ.

C/EBPβ and C/EBPδ are transiently expressed and function at the early stage of terminal adipogenesis by sensing adipogenic stimuli and initiating expression of PPARγ and C/EBPα (Cao et al., 1991; Yeh et al.,...
C/EBPβ is thought to initiate mitotic clonal expansion of preadipocytes and to later coordinate the transcriptional network by turning on C/EBPα and PPARγ (Tang et al., 2003). In the present study, we found that INH reduces the expression of C/EBPβ and C/EBPδ in the early stage of adipogenesis. Because the expression of Cebpβ at the early stage of adipogenesis is highly dependent on Nrf2, which binds to an ARE site in the Cebpβ promoter and increases its transcription (Hou et al., 2012), the suppression of ARE by INH may lead to reduction of C/EBPβ. It has been reported that multiple nuclear factors, including CREB, activating transcription factors and silencing mediator for retinoid and thyroid hormone receptors, which are all implicated in Nrf2-mediated antioxidant response (Bakin et al., 2005; Brown et al., 2008; He et al., 2001; Kang et al., 2005; Katoh et al., 2001; Ki et al., 2005), may regulate and/or interact with C/EBPβ or C/EBPδ (Belmonte et al., 2001; Choy and Derynck, 2003; Fox et al., 2006; Zhang et al., 2004). Thus, Nrf2 may coordinate with these adipogenic factors and regulate the early stage induction of C/EBPβ and/or C/EBPδ expression.

Multiple transcription factors, including Nrf2, activator protein 1, small Maf proteins, and other bZIP proteins, are involved in the transcriptional regulation of ARE-dependent genes (Motohashi et al., 1995).
Fig. 3. INH suppresses adipogenesis in human ADSCs. (A) Lipid accumulation in differentiated human ADSCs. Cells were cultured to 95% confluence and differentiated for 5 days using the DMI protocol. INH was added during Days 1 and 2 of differentiation. Following differentiation, the images of cells were captured before (left panels) and after (right panels) ORO staining to visualize lipid accumulation (20×). (B) mRNA expression of Pparγ. Control cells were differentiated by using the DMI protocol for 5 days. INH (2 or 10 mM), INH at indicated concentrations was added during Days 1 and 2 of DMI-induced differentiation. n = 3. *p < 0.05 vs. Control.

Fig. 4. Effects of INH on ARE activity and expression of C/EBPβ and C/EBPδ during early stage of adipogenesis in 3T3-L1 cells. (A) INH inhibits ARE activity in 3T3-L1 cells stably transduced with ARE–luciferase reporter. DMI, cells were differentiated by using the DMI protocol for the indicated time; DMI + INH, INH (5mM) was added 4h prior to the DMI treatment and kept in the medium during the entire differentiation process. Veh cells were maintained in growth medium without DMI. The activity of ARE–luciferase was normalized by protein levels. Veh cells were maintained in growth medium without DMI. n = 3. *p<0.05 vs. DMI alone at the same differentiation time; #p<0.05 vs. DMI with Veh. (B–C) mRNA (B) and protein (C) expression of C/EBPβ and C/EBPδ in normal 3T3-L1 cells. Cells were treated as in (A), n = 3–5. (D) Quantification of (C). *p<0.05 vs. DMI alone at the same differentiation time; #p<0.05 vs. DMI with Veh.
2002). To determine the molecular mechanisms by which INH suppresses the expression of C/EBPα and C/EBPβ, we also determined the effects of INH on their key transcriptional regulators during the early stage of adipogenesis in 3T3-L1 cells. Surprisingly, INH had no effect on the protein expression of p-CREB, C/EBP homologous protein (CHOP), MafG/K, c-Jun or c-FOS in the early stage of DMI-induced adipogenesis, despite that dramatic induction of p-CREB, Nrf2 and c-FOS or downregulation of CHOP occurred in response to DMI challenge (Fig. S2). These findings revealed that INH does not affect the expression of these proteins, indicating that the suppression of INH on ARE activity is independent of the availability of these proteins. Recently, Metushi et al. (2012) reported that INH and its reactive metabolites covalently bind to multiple hepatic proteins, suggesting that INH may directly interfere with protein function in general. Future studies will be directed towards whether INH or its metabolites may bind to Nrf2 and/or other ARE-related bZIP proteins leading to suppression of their binding activity towards the AREs.

Our recent studies demonstrated that reduction of adipogenesis due to Nrf2 deficiency appears to be associated with impaired adipose function in ob/ob mice, which leads to ectopic lipid accumulation in circulation and hyperglycemia (Xue et al., 2013). In agreement with this finding, it has been shown that INH treatment of phenobarbitone-pretreated rabbits resulted in a reduction in total lipid, triglyceride and cholesterol in adipose tissues, but hyperlipidemia and hepatosteatosis (Krishnamoorthy and Karthikeyan, 1991). In addition, a case report by Katoh et al. (2009) indicated that antitubercular drugs, including INH, tend to promote a worsening of glycemic control, which can be improved by rosiglitazone. Thus, it is highly likely that impaired glucose and lipid homeostasis associated with INH treatment may originate from suppressed adipogenesis by INH as demonstrated in the present study. This new aspect of INH-induced complications needs further study.

In summary, the data presented here clearly demonstrated that INH suppresses ARE activity in mouse and human cells leading to reduced expression of many antioxidant genes. INH concentration-dependently suppresses adipogenesis in mouse preadipocytes and human ADSCs. However, the connection between reduced ARE activity and suppression of adipogenesis by INH still need further investigation. Given the critical role of adipogenesis in adipose development and function, a better understanding of the effect of INH on glucose and lipid homeostasis is needed.

Conflict of interest

The content is solely the responsibility of the authors. PX, TZ, QZ, MEA and JP are employees of The Hamner Institutes for Health Sciences. The Hamner is a 501(c)3 not-for-profit organization that includes funding from the American Chemical Society. MEA and JP are employees of The Hamner Institutes for Health Sciences.

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Appendix A. Supplementary data

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References


