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Dysregulation of Δ^4-3-oxosteroid 5β-reductase in diabetic patients: Implications and mechanisms

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ABSTRACT

Aldo-keto reductase family 1 member D1 (AKR1D1) is a Δ^4-3-oxosteroid 5β-reductase required for bile acid synthesis and steroid hormone metabolism. Both bile acids and steroid hormones, especially glucocorticoids, play important roles in regulating body metabolism and energy expenditure. Currently, our understanding on AKR1D1 regulation and its roles in metabolic diseases is limited. We found that AKR1D1 expression was markedly repressed in diabetic patients. Consistent with repressed AKR1D1 expression, hepatic bile acids were significantly reduced in diabetic patients. Mechanistic studies showed that activation of peroxisome proliferator-activated receptor-α (PPARα) transcriptionally down-regulated AKR1D1 expression *in vitro* in HepG2 cells and *in vivo* in mice. Consistently, PPARα signaling was enhanced in diabetic patients. In summary, dysregulation of AKR1D1 disrupted bile acid and steroid hormone homeostasis, which may contribute to the pathogenesis of diabetes. Restoring bile acid and steroid hormone homeostasis by modulating AKR1D1 expression may represent a new approach to develop therapies for diabetes.

1. Introduction

Aldo-keto reductase family 1, member D1 (AKR1D1) is a Δ^4-3-oxosteroid 5β-reductase, which catalyzes the reduction of steroids with a 3-oxo-4-ene structure in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) as a hydride donor (Kondo et al., 1994). AKR1D1 is required for cholesterol metabolism into bile acids. In the bile acid synthesis pathway, AKR1D1 reduces the double bond in the A ring of the bile acid intermediates to eventually produce primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA) (Russell, 2003; Jin et al., 2014). AKR1D1 is also involved in metabolism and clearance of steroid hormones with 3-oxo-4-ene structure including glucocorticoids (Jin et al., 2014; Chen and Penning, 2014). Since 5β-reduction is a common transformation and major inactivation pathway for steroid hormones, and AKR1D1 is the only enzyme in humans capable of catalyzing a 5β-reduction in those steroids, AKR1D1 plays a critical role in regulating and maintaining the homeostasis of steroid hormones, including glucocorticoids. Therefore, AKR1D1 plays critical roles in bile acid synthesis and steroid hormone metabolism and inactivation.

Bile acids are actively participated in regulating metabolism and energy expenditure. A large body of evidence from human clinical (Bennion and Grundy, 1977; de Leon et al., 1978; Steiner et al., 2011; Zhang et al., 2015) and animal model (Nervi et al., 1978; Uchida

The abbreviations used are: AKR1D1, aldo-keto reductase family 1, member D1; BMI, body mass index; BSEP, bile salt export pump; CA, cholic acid; CDCA, chenodeoxycholic acid; CPT1A, carnitine palmitoyltransferase-1A; CYP7A1, cholesterol 7α-hydroxylase or cytochrome P450 family 7 subfamily A member 1; CYP7B1, cytochrome P450 family 7 subfamily B member 1; CYP8B1, sterol 12α-hydroxylase or CYP family 8 subfamily B member 1; CYP27A1, cytochrome P450 family 27 subfamily A member 1; DCA, deoxycholic acid; FXR, farnesoid x receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LCA, lithocholic acid; LXR, liver x receptor; LC-MS/MS, liquid chromatography tandem-mass spectrometry; LLOD, low limit of quantification; LLOQ, low limit of detection; MRP3, multidrug resistance-associated protein 3; MRP4, multidrug resistance-associated protein 4; NADPH, nicotinamide adenine dinucleotide phosphate; NTCP, sodium-taurocholate cotransporting polypeptide; OATP1B1, organic anion-transporting polypeptide member 1B1; PGZN, pioglitazone; PPARα, peroxisome proliferator-activated receptor alpha; SEM, standard error of mean; TGR5, Takeda G-protein receptor 5; UDCA, ursodeoxycholic acid.

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et al., 1979) investigations demonstrate that bile acid homeostasis is disrupted in patients with metabolic disease and diabetes while moderating bile acid enterohepatic circulation has beneficial effects on diabetes (Prawitt et al., 2014; Flynn et al., 2015; Ferrannini et al., 2015). On the other hand, steroid hormones, especially glucocorticoids, play important roles in regulating body metabolism and energy expenditure (Vegiopoulos and Herzig, 2007; de Guia et al., 2014). Studies with human and various rodent models have linked dysregulation of glucocorticoids to the development of metabolic disease and diabetes (Pivonello et al., 2010; Chan et al., 2003; Perez et al., 2014).

As a key player in bile acid synthesis and steroid hormone metabolism, our current understanding on AKR1D1's roles in metabolic disease and diabetes is limited (Valanejad et al., 2017). In this study, we found that AKR1D1 expression was markedly repressed in diabetic patients. In line with repressed AKR1D1 expression, hepatic bile acid levels were significantly reduced in diabetic patients compared with non-diabetic control subjects. Mechanistic studies revealed that activation of peroxisome proliferator-activated receptor-α (PPARα) markedly repressed AKR1D1 expression *in vitro* and *in vivo*. Furthermore, it was revealed that PPARα signaling was enhanced in diabetic patients, which provides an explanation for the repressed expression of AKR1D1 in diabetic patients.

2. Materials and methods

2.1. Chemicals and supplies

CDCA, CA, deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), glycine or taurine conjugated bile acids, dimethyl sulfoxide (DMSO), DNA oligonucleotides, fetal bovine serum (FBS), charcoal-stripped FBS, pioglitazone (PGZN) and insulin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Luciferase assay kits were from Promega (Madison, WI, USA). Restriction enzymes were from New England BioLabs (Ipswich, MA, USA). Activated charcoal was from Fisher Scientific (Pittsburgh, PA, USA). GW4064, GW7647, GW3965 and GW0742 were purchased from Tocris Bioscience (Minneapolis, MN, USA). Recombinant human AKR1D1 protein was purchased from Novus Biologics (Littleton, CO, USA).

2.2. Liver samples

Liver samples: Twenty-two liver tissues of type 2 diabetes mellitus patients were obtained from Sckisui XenoTech (Kansas City, KS, USA). The quality of those liver tissues were ensured with initial intent for liver transplantation. Twenty non-diabetic normal control liver samples were obtained through the Cooperative Human Tissue Network (CHTN). The quality of the liver tissues provided by CHTN were ensured through the prospective collection model as the quality management system (Grizzle et al., 2015). The characteristics of individual liver tissues from diabetic and non-diabetic control subjects were provided in Tables 1 and 2. The protocol for using human tissues was approved by the Institutional Review Board (IRB) at the University of Rhode Island (URI).

2.3. Plasmid constructs

Human AKR1D1 promoter reporter phAKR1D1(-5.0 kb) was constructed by cloning the 5 kb promoter region into the pGL4.10 luciferase vector (Promega). The 5 kb promoter was PCR-amplified by forward (5′-TAAGATGCTAATCGTAATCCCCAGGGTAAC-3′) and reverse primer (5′-GGAGTGATTCTGAACACCACAGGGAGTCT-3′) using human genomic DNA as templates. Expression plasmids for human farnesoid x receptor-α2 (FXRα2) was provided by Dr. Matthew Stoner. Expression plasmids for human FXRα1, PPARα, PPARβ, PPARγ and liver x receptor-α (LXRα) were kindly provided by Dr. Bingfang Yan at URI.

2.4. Mice and treatments

Eighteen CD-1 mice were randomly divided into three groups. One group of mice were treated with PPARα agonist GW7647 (10 mg/kg). The second group were treated with FXR agonist GW4064 (10 mg/kg). The third group were treated with vehicle propanediol as controls. Mice were injected i.p. twice a day for 3 days. Twelve hours after the last injection, animal were euthanized and liver tissues were harvested and processed for gene expression analysis. The animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at URI.

2.5. HepG2 cells and treatments

HepG2 cells seeded in 12-well plates were treated with GW4064 (1 μM), GW7647 (10 μM), PGZN (10 μM) and insulin (100 ng/ml) in phenol-red free medium containing 1% charcoal striped FBS for 30h, followed by analysis of gene expression.

2.6. TaqMan quantitative real-time PCR

Total RNA isolation from liver tissues (human and mice) or HepG2 cells and subsequent TaqMan real-time PCR were carried out as described (Song et al., 2014). Transcript levels of AKR1D1, FXR, PPARα, cholesterol 7α-hydroxylase or cytochrome P450 family 7 subfamily A member 1 (CYP7A1), sterol 12α-hydroxylase (CYP8B1), bile salt export pump (BSEP), sodium-taurocholate cotransporting polypeptide (NTCP), organic anion-transporting polypeptide member 1B1 (OATP1B1), multidrug resistance-associated protein 3 (MRP3), MRP4, cytochrome P450 family 27 subfamily A member 1 (CYP27A1), cytochrome P450 family 7 subfamily B member 1 (CYP7B1) and carnitine palmitoyltransferase-1A (CPT1A) were normalized against β-actin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) levels according to the treatments or conditions, which had minimal effects on β-actin or GAPDH (Kroetz et al., 1998; Ma et al., 2013; de la Monte et al., 2011; Zhou et al., 2008; Biswas et al., 2013). Validated TaqMan PCR probes and master mixtures were obtained from Applied Biosystems (Foster City, CA, USA).

2.7. Analysis of bile acids in human liver by liquid chromatography tandem-mass spectrometry (LC-MS/MS)

Bile acid extraction from human liver tissues and subsequent quantification by LC-MS/MS were carried out as described with some modifications (Alnouti et al., 2008). An Acquity UPLC from Waters connected to a Xevo TQ MS mass spectrometry was used for quantification. The system was controlled with MassLynx™ software (V 4.1) and data was processed using TargetLynx™ tool.

For preparation of liver tissue extracts for quantification of bile acids, liver samples were homogenized on ice in 1:4 w/v 50% methanol (Fisher Scientific, AC61513). After addition of 10 μl of mifeprestone internal standard and 2 ml of ice-cold alkaline acetonitrile (5% ammonium hydroxide in acetonitrile) (Fisher Scientific), samples were vortexed and shaken continuously for 1 h at 4 °C. Samples were then centrifuged at 4600 × g for 20 min at 4 °C, supernatants were collected. The pelleted samples were extracted again with 1 ml of ice-cold alkalinizede acetonitrile and the supernatants were collected. The two supernatants were pooled and then evaporated using a Thermo Scientific SpeedVac system,

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