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Original Contribution

The oxidative stress mediator 4-hydroxynonenal is an intracellular agonist of the nuclear receptor peroxisome proliferator-activated receptor-β/δ (PPARβ/δ)

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Abstract

Liver insufficiency and damage are major causes of death and disease worldwide and may result from exposure to environmental toxicants, specific combinations or dosages of pharmaceuticals, and microbial metabolites. The generation of reactive intermediates, in particular 4-hydroxynonenal (4-HNE), is a common event in liver damage caused by a variety of hepatotoxic drugs and solvents. The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that are involved in the transcriptional regulation of lipid metabolism as well as other biological functions. Importantly, we have observed that the PPARβ/δ$^{-/-}$ mouse is more susceptible to chemically induced hepatotoxicity than its wild-type counterpart, and our objective in this study was to elucidate the mechanism(s) by which PPARβ/δ confers protection to hepatocytes. We hypothesized that PPARβ/δ plays a protective role by responding to toxic lipids and altering gene expression accordingly. In support, oxidized-VLDL and constituents including 13-*S*-hydroxyoctadecadienoic acid (13-S-HODE) and 4-HNE are PPARβ/δ ligands. A structure–activity relationship was established where 4-HNE and 4-hydroperoxynonenal (4-HpNE) enhanced the activity of the PPARβ/δ subtype while 4-hyroxyhexenal (4-HHE), 4-oxo-2-Nonenal (4-ONE), and *trans*-4,5-epoxy-2(*E*)-decenal did not activate this receptor. Increasing PPARβ/δ activity with a synthetic agonist decreased sensitivity of hepatocytes to 4-HNE and other toxic agents, whereas inhibition of this receptor had the opposite result. Gene expression microarray analysis identified several important PPARβ/δ-regulated detoxification enzymes involved in 4-HNE metabolism that are regulated at the transcript level. This research established 4-HNE as an endogenous modulator of PPARβ/δ activity and raises the possibility that agonists of this nuclear receptor may be utilized to prevent or treat liver disease associated with oxidative damage.

Introduction

Greater than 2.2 million hospitalized Americans suffer adverse drug reactions each year, with liver toxicity presenting as the most common adverse effect, and approximately 100,000 individuals die unintentionally from administration of medications [1,2]. Many additional cases of liver failure occur due to acute, chronic, and degenerative disease processes, including those related to acetaminophen overdose, alcohol consumption, and solvent exposures. Reactive oxygen intermediates (ROI) elicit oxidative decomposition of polyunsaturated fatty acids (i.e., lipid peroxidation), leading to the formation of a complex mixture of aldehydic end products, including malondialdehyde (MDA), 4-HNE, and other alkenals [3]. These aldehydic molecules have been considered the ultimate mediators of toxic effects elicited by oxidative stress but may also affect cellular function at nontoxic levels via signal transduction, gene expression, and cell proliferation. Although the overt toxicity

Abbreviations: 4-HNE, 4-hydroxynonenal; 13-S-HODE, 13-*S*-hydroxyoctadecadienoic acid; PPARs, peroxisome proliferator-activated receptors; ROI, reactive oxygen intermediates; MDA, malondialdehyde; LpL, lipoprotein lipase; TTA, tetradecylthioacetic acid; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; RT-PCR, reverse transcriptase polymerase chain reaction.

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caused by aldehydic end products is due primarily to covalent binding to cellular macromolecules, the effects on signal transduction are not well characterized. Since millions of individuals suffer adverse drug reactions each year it is important to understand how the cell responds to intracellular insults such as production of ROI and 4-HNE.

The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that exist as three subtypes (α, β/δ, and γ), which exhibit tissue-specific expression, preferential ligand recognition, and distinct biological functions [4–9]. Although important as targets of pharmaceutical intervention, there is increasing evidence that the biological niche occupied by the PPARs is that of a receptor for fatty acid and their metabolites. Of the three PPAR genes (α, β/δ, and γ), the PPARβ/δ isoform is the least well studied in terms of its biological functions and endogenous ligands. PPARβ/δ plays an important role in differentiation of epithelial tissues, fatty acid catabolism in skeletal muscle, improvement of insulin sensitivity, attenuated weight gain, and elevated HDL levels [10]. Emerging evidence suggests that the presence of this receptor is important in ameliorating the effects of hepatotoxicants. For example, histological examination of liver and analysis of markers of overt damage to this organ (serum GPT) after treatment with the xenobiotics azoxymethane (AOM), arsenic, or carbon tetrachloride demonstrated that the extent of liver toxicity in PPARβ/δ-null mice was more severe than in wild-type mice.[2] While it is remotely possible that the metabolic fate of these hepatotoxicants could be influenced by PPARβ/δ, it is more likely that regulation of oxidative stress underlies the protective role of this receptor in liver. These chemicals share a common mechanism of overt toxicity via production of ROI and oxidized lipid intermediates. For example, CCl_4 affects eicosanoid pathways [11,12] and increases circulating prostaglandin E_2 (PGE_2) levels [13] and 4-HNE and 4-HNE-protein adducts [3,14,15]. The purpose of this study was to determine the extent to which oxidized lipids and their metabolites interact with PPARβ/δ and influence gene regulation. We hypothesized that PPARβ/δ acts as an oxidative stress sensor in hepatocytes and, upon interaction with products of lipid peroxidation, regulates detoxification genes accordingly to ameliorate the toxic insult.

One possible explanation for the increased susceptibility of PPARβ/$\delta^{-/-}$ mice to hepatotoxicity is that oxidative damage increases the production of an endogenous ligand for PPARβ/δ. This putative agonist would in turn stimulate lipid metabolism and degradation of lipid peroxidation intermediates. PPARs are well recognized as transcriptional regulators of lipid metabolism, transport, storage, and other activities [16]. In the absence of PPARβ/δ the signaling cascade would be disrupted and accumulation of toxic lipids such as 4-HNE would result. If our hypothesis were correct, endogenous ligands of PPARβ/δ should include oxidized lipids, in particular those derived from fatty acids. In support, we discovered that oxidized-VLDL and constituents including 13-S-HODE and 4-HNE are PPARβ/δ agonists. In addition, modulating PPARβ/δ activity, either by activation with synthetic PPARβ/δ-selective agonist tetradecylthioacetic acid (TTA) or inhibition with PPAR pan-antagonist GW9662 [17], affects the sensitivity of hepatocytes to 4-HNE and other toxic agents. This research raises the possibility that PPARβ/δ agonists may be utilized to prevent or treat liver disease associated with the generation of ROIs.

[2] W. Shan et al., manuscript in preparation.

Materials and methods

Reagents

VLDL (human plasma) was purchased from Calbiochem (La Jolla, CA), LPL was purchased from Sigma (lyophilized powder) and reconstituted in PBS (10 mg/mL), and 13-S-HODE, 13-S-HpODE, 4-HHE, 4-ONE, *trans*-4,5-epoxy-2E-decenal, 4-HpNE, and 4-HNE were purchased from Cayman Chemical (Ann Arbor, MI) and used without further purification. The semienzymatic synthesis and purification of some of the linoleic and arachidonic acid oxidation products such as 9-HODE, 12-HpODE, 5-HETE, 9-HETE, 12-HETE, 15-HETE, 5-HpETE, 15-HpETE, 5,15-diHpETE, and 5,6-diHETE were performed as described [18]. The authenticity of each of the lipid mediators was confirmed using cochromatography as well as gas chromatography-mass spectrometric experiments. UV-visible spectroscopy was used to determine their respective concentrations, and the compounds were reconstituted in anhydrous ethanol to the desired concentration. The compounds were used within 30 min of reconstitution in ethanol.

Plasmids

Plasmids used in reporter assays including pM/mPPAR-α, -β, and -γ, pBK/mPPAR-α, -β, and -γ (murine), ACO Luciferase, and pFR-luciferase (Promega) have been described elsewhere [19].

Cell culture

3T3-L1 preadipocyte cells were grown in standard high-glucose Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. MuSH cells were grown in standard α-MEM containing 10% FBS. Cells were treated as described in the figure legends.

Transient transfection and treatment

Cells were counted using a hemocytometer following staining with trypan blue solution (0.4%) (Sigma) and plated onto 10-cm dishes at densities between 600,000 and 800,000 cells/dish for transfection. Transfections were performed at 37°C for 6 h using 12 μg of the appropriate Gal4-PPAR ligand binding domain (LBD) plasmid (pM/mPPAR-α, -β, or -γ) containing a Luciferase reporter, and 24 μg Lipofectamine (Invitrogen). Transfection media were then replaced with fresh high-glucose DMEM after washing the dishes with PBS and the cells were allowed to recover overnight.