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Elevated adiponectin prevents HIV protease inhibitor toxicity and preserves cerebrovascular homeostasis in mice

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ABSTRACT

HIV protease inhibitors are key components of HIV antiretroviral therapies, which are fundamental in the treatment of HIV infection. However, the protease inhibitors are well-known to induce metabolic dysfunction which can in turn escalate the complications of HIV, including HIV associated neurocognitive disorders. As experimental and epidemiological data support a therapeutic role for adiponectin in both metabolic and neurologic homeostasis, this study was designed to determine if increased adiponectin could prevent the detrimental effects of protease inhibitors in mice. Adult male wild type (WT) and adiponectin-overexpressing (ADTg) mice were thus subjected to a 4-week regimen of lopinavir/ritonavir, followed by comprehensive metabolic, neurobehavioral, and neurochemical analyses. Data show that lopinavir/ritonavir-induced lipodystrophy, hypoadiponectinemia, hyperglycemia, hyperinsulinemia, and hypertriglyceridemia were attenuated in ADTg mice. Furthermore, cognitive function and blood–brain barrier integrity were preserved, while loss of cerebrovascular markers and white matter injury were prevented in ADTg mice. Finally, lopinavir/ritonavir caused significant increases in expression of markers of brain inflammation and decreases in synaptic markers in WT, but not in ADTg mice. Collectively, these data reinforce the pathophysiologic link from metabolic dysfunction to loss of cerebrovascular and cognitive homeostasis; and suggest that preservation and/or replacement of adiponectin could prevent these key aspects of HIV protease inhibitor-induced toxicity in clinical settings.

1. Introduction

Combination HIV antiretroviral therapy restricts viral replication, raises CD4 cell counts, prevents opportunistic infections, and improves/extends the lifespan and healthspan of people living with HIV/AIDS [1]. In spite of these revolutionary effects, it is well known that these drugs, particularly HIV protease inhibitors, have significant metabolic complications, fostering the development of dyslipidemia, insulin resistance, and lipodystrophy [2,3]. This iatrogenic sequela undermines patient health and limits ART compliance [4], and can also predispose patients to cognitive impairment and other neurologic complications [5–9]. Thus, these metabolic co-morbidities must be clinically managed to preserve quality of life and maintain self-care independence for people living with HIV/AIDS. Unfortunately, however, current pharmacologic strategies have produced only limited success in clinical settings [10]. For example, the insulin-sensitizing drug metformin reduces insulin resistance in HIV patients [11,12], but does not improve hyperlipidemia [12] and may actually accelerate lipodystrophy [13]. Thiazolidinediones (TZDs) show a similar dichotomous pattern with improvement in insulin sensitivity [11,12], but increased hyperlipidemia [11,12] and accelerated bone loss [14]. Tesamorelin, a recently approved synthetic human growth hormone-releasing hormone (hGHRH) analogue designed to treat HIV lipodystrophy, has been shown to decrease abdominal fat accumulation, improve glucose homeostasis [15], and even preserve cognition in adults with mild cognitive impairment [16]. However, studies have also shown that hGHRH can decrease subcutaneous fat mass, as well as induce arthralgia and edema [17]. Thus, new therapeutic approaches to significantly and successfully mitigate metabolic co-morbidities in HIV patients are needed.

In this context, remedies that prevent lipodystrophy and/or replicate adipocyte function in the face of lipodystrophy could provide novel and complementary strategies to maintain metabolic and neurologic function in people living with HIV/AIDS. Adipocytes orchestrate aspects of physiology via secretion of adipokines [18]; and in particular, adiponectin may be fundamental for optimal health. In terms of metabolic homeostasis, adiponectin is known to modulate glucose and fatty acid metabolism, inflammation, and vascular tone [19]. Adiponectin

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also has both vasculoprotective and neuroprotective properties [20–22], and indeed, hypoadiponectinemia predicts cognitive impairment [23] and decreased hippocampal volume [24] in humans. In specific relation to HIV, serum adiponectin levels are decreased in HIV patients [25–27], and correlate inversely with cognitive dysfunction in mice treated with HIV protease inhibitors [28]. Indeed, adiponectin administration has been shown to mitigate protease inhibitor-induced dyslipidemia in mice [29], suggesting that hypoadiponectinemia may drive, at least in part, the metabolic derangements associated with HIV protease inhibitors. Collectively these data support investigation into adiponectin-based therapies in the context of HIV antiretroviral therapy to support metabolic function and curtail the development of HIV-associated neurocognitive disorders. To determine the ability of adiponectin to prevent the adverse metabolic and neurologic effects of protease inhibitors, adult male wild type (WT) and adiponectin-overexpressing (ADTg) mice were subjected to a clinically relevant regimen of lopinavir/ritonavir, followed by comprehensive metabolic, neurobehavioral, and biochemical analyses.

2. Materials and methods

2.1. Animal treatments

The Institutional Animal Care and Use Committee at the Pennington Biomedical Research Center approved all experimental protocols, which were compliant with NIH guidelines on the use of experimental animals. ADTg mice on a C57Bl/6 background were generated as described in [30]. These mice express an aP2 promoter-driven transgene encoding a truncated form of adiponectin, leading to chronic, ~2 to 2.5-fold induction of secretion of oligomeric adiponectin complexes [30]. 6–8 month-old male ADTg and wild-type (WT) littermate control mice generated from local breeding colonies were housed in standard caging with 12:12 light:dark cycle and ad libitum access to food and water. Lopinavir/ritonavir (Kaletra®, Abbott Laboratories), was diluted in a vehicle of 10% ethanol/15% propylene glycol, and mice received daily administration of vehicle or lopinavir/ritonavir at 150/37.5 mg/kg via daily oral gavage for 4 weeks as previously described [28,31,32]. The dose was devised based on dosing guidelines for daily oral lopinavir/ritonavir in adult HIV patients (800/200 total mg or 10/2.5 mg/kg), and on body surface area (BSA) normalization factors [33], which translate 10 mg/kg in humans to approximately 125 mg/kg in mice. Previous UPLC-MRM-MS measurements of serum lopinavir 4 h after intraperitoneal injection of combined lopinavir/ritonavir show that that serum lopinavir in mice (3–18 μg/ml) approximates Abbott's reported C_{max} for lopinavir of 9.8 + 3.7 μg/ml 4 h after administration to adult HIV-positive patients [31].

Body weight and composition (measured using a Bruker minispec LF90 time domain NMR analyzer, Bruker Optics, Billerica MA) were measured regularly throughout lopinavir/ritonavir exposure. Fasting blood glucose was measured in tail blood using a glucometer (Ascensia Elite, Bayer, Mishawaka, IN). After cognitive testing, all mice were humanely euthanatized after a brief (6 h) fast, and blood, cerebral spinal fluid (CSF), and brain were collected. Data were compiled from 3 separate cohorts of mice.

2.2. Fear conditioning memory task

Each mouse was individually evaluated for fear conditioning using an automated, video-based fear conditioning system (Med-Associates, St. Albans, VT) as described previously [32,34]. The apparatus consists of a "startle chamber" used on days 1 and 2, which is an 8 × 15 × 15-cm acrylic and wire mesh cage located within a custom designed 90 × 70 × 70 ventilated sound-attenuating chamber, and the unique context is reinforced with an anise-based scent applied to each cage before testing. Animal movement within the apparatus results in displacement of an accelerometer (model U321AO2; PCB Piezotronics, Depew, NY, USA). Acquisition of fear conditioning on day 1 consists of 5 min acclimation to the startle chamber, followed by five consecutive 30 s auditory stimuli (85 db, 4 kHz) co-terminating with a mild footshock (0.5 mA × 1 s), with 30 s recovery periods between tones. On day 2, mice return to the same chambers, but no stimuli are applied to evaluate freezing responses to context. On day 3, mice are placed in an entirely separate chamber located in a different room to remove all contextual cues, and after 5 min habituation, a continuous tone (85 db, 4 kHz) is applied for 5 min. Freezing behavior is recorded as a measure of memory of the conditioned response to the tone.

2.3. Measurement of blood–brain barrier permeability

Sodium fluorescein (NaF, 376 Da) was used to assess the BBB permeability using established protocols [35]. Briefly, mice received an intravenous injection of PBS (150 μL) containing sodium fluorescein (NaF, 2 mg/mL; Sigma). Exactly 30 min later, blood was collected from the right atrium, mice were immediately perfused with 15 mL ice-cold PBS, and brain tissues were collected and kept at 4 °C. Weighed sections of the cerebral cortex and samples of serum were homogenized in 0.5 M borate buffer (pH 10) and centrifuged at 800 ×*g* for 15 min at 4 °C. Supernatants were mixed with ethanol and then centrifuged (15,000 ×*g*) for 20 min at 4 °C. Supernatant brain and serum Na-F concentrations were measured with a fluorimeter at 460 nm excitation and 515 nm emission within a linear range of standards of known concentrations and tissue/serum ratio of fluorescence was determined.

2.4. Clinical chemistry

Whole blood was collected by cardiac puncture of terminally anesthetized mice, and plasma was collected and either analyzed immediately or aliquoted and stored at −80 °C. Levels of total cholesterol, triglycerides, and non-esterified fatty acids (NEFA) in plasma were measured calorimetrically using commercially available kits (Wako Chemicals, Richmond, VA). Adiponectin and insulin levels in plasma and CSF were evaluated by ELISA in accordance with the manufacturer's assay protocol (R&D Systems, Minneapolis, MN).

2.5. Western blot

Tissue samples were homogenized and processed for Western blot with chemiluminescence as described in previous reports [36]. Blots were processed using the following primary antisera: anti-claudin-5 (1:400, Abcam Inc., Cambridge, MA), anti-ZO-1 (1:100, Abcam Inc.), anti-occludin (1:8000, Abcam Inc.), anti-MMP2 (1:1000, Abcam Inc.), anti-MMP-9 (1:1000, Abcam Inc.), anti-synapsin 1 (1:10,000, Thermo Fisher Scientific, Pittsburg, PA), anti-phospho(S553)-synapsin 1 (1:10,000, Abcam Inc.), anti-synapse associated protein 97 (1:2500, Abcam Inc.), anti-GFAP (1:5000, Abcam Inc.); anti-Iba-1 (1:500, Wako Chemicals USA Inc., Richmond, VA), and anti-tubulin (1:1000, Wako Chemicals USA Inc.). To ensure accurate quantification across multiple blots, samples from all treatment groups (vehicle and lopinavir/ritonavir in both WT and ADTg mice) were included in each individual blot. Data were first calculated as a ratio of expression over tubulin expression, which was included as an internal loading control, and then expression in lopinavir/ritonavir-treated mice was calculated and presented as percent expression in vehicle-treated mice.

2.6. Luxol fast blue stain

For histological examination, hemibrains were rapidly collected from mice and immersed in 10% neutral buffered formalin for 24–48 h and then processed for paraffin embedding. 6 μm mid-sagittal sections containing the anterior (genu) corpus callosum at the level of the lateral septal nuclei, medial to the lateral ventricle were selected, and following standard dewaxing and rehydration, tissue sections were immersed