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Acute Statin Administration Reduces Levels of Steroid Hormone Precursors

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Abstract

Cholesterol-lowering statin drugs are used by approximately 25 % of US adults 45 years of age and older and frequency of use is even higher among the elderly. Cholesterol provides the substrate for steroid hormone synthesis and its intracellular concentrations are tightly regulated. Our aim was to evaluate whether statin use acutely changes the circulating levels of cortisol, other glucocorticoid precursor molecules and their metabolites. Fourteen subjects not taking statins were administered a single oral dose (2 mg) of pitavastatin. Blood samples collected at baseline and 24 h post-treatment were analyzed for plasma cholesterol and steroid hormone profile. A parallel study in mice entailed the administration of atorvastatin (10 mg/kg) via orogastric delivery for three consecutive days. Cholesterol and corticosterone levels were quantified at baseline and at 1-day and 1-week post-treatment. Several precursor molecules in the steroidogenic pathway (corticosterone, cortisone, and 11-deoxycortisol) were significantly decreased 24 h after administration of a single dose of pitavastatin in human study subjects. Their circulating cholesterol concentrations were unchanged. In mice, there were no significant differences in serum cholesterol or corticosterone at 1-day or 1-week post-treatment compared to both pre-treatment baseline levels and control group levels. We conclude that acute dysregulation of the production of certain glucocorticoid precursor molecules was observed after a single treatment with a lipophilic statin drug. This may be of clinical relevance for individuals with underlying or subclinical adrenal insufficiency.

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Conflict of Interest

The authors declare that they have no conflict of interest. Dr. Stratakis' laboratory has received research grants from Pfizer Inc. for subjects unrelated to the investigation reported here.

Keywords

glucocorticoids; cholesterol; statin

Introduction

Cholesterol is the common and unique substrate for the initial step of steroidogenesis in the adrenal glands, gonads, and placenta [1]. In human adrenocortical cells, cholesterol is supplied via two distinct pathways: uptake of circulating cholesterol from plasma low-density lipoproteins (LDL), and to a lesser degree, de novo cholesterol production within the adrenal gland [2, 3]. To maintain eucortisolemia, ACTH tightly regulates cholesterol in adrenocortical cells, via direct stimulation/inhibition of the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) as well as LDL receptor expression [4].

The potential interference of statins (HMG-CoA inhibitors) in steroidogenesis has been hypothesized since their discovery, but previous studies have been inconclusive about their effect on cortisol production [5–7]. Most reported studies focused on the established effects of statins after at least two-week administration; statins are used for their lipid-lowering and other effects in patients with coronary artery disease and diabetes [8, 9], and several side effects have been noted [10–15]. We designed a small-scale study in a cohort of individuals not taking statins and in mice to test the hypothesis that statins could elicit acute changes in the steroidogenic pathway of the adrenal cortex.

Subjects and Methods

Clinical protocol

Participants were recruited by the National Human Genome Research Institute (NHGRI), National Institutes of Health (NIH) under protocol 14-HG-0203, aimed to examine the effect of gene variants on statin absorption and metabolism. Volunteers (n = 14) were 18–55 years old, not currently taking statins or cholesterol-lowering drugs, or willing and safely able to discontinue statins ten days prior to the study as determined by a cardiologist. Participants were excluded if they were pregnant, breastfeeding; or had history of easy bleeding or bruising; liver, neuromuscular, or thyroid disease; organ transplant, chest pain, myocardial infarction or stent placed in the past year. Informed consent documents were signed upon enrollment and clinical studies were approved by the NHGRI Institutional Review Board.

All participants were administered a single 2 mg dose of pitavastatin (08:00 h $\pm$ 30 min). Blood samples were collected immediately after placement of an IV catheter that was left in place for 24 h, except in two participants who did not elect IV collection. The data presented utilized blood samples collected at baseline (T = 0) and 24 h post-statin administration.

Murine study

Mouse studies and procedures were approved by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Institute of Animal Care and Use

Committee (IACUC). Fourteen adult male C57/Bl6 wild-type mice (20–26 wk old) were randomized to either the treatment group that was administered 10 mg/kg BW atorvastatin calcium (USP) in 0.9 % sterile saline or control group (0.9 % sterile saline) (n = 7/group). Treatment or vehicle was delivered by oral gavage (500 µl) for three consecutive days (09:00 h). Baseline, post-treatment, and follow-up blood samples were collected 1 week prior to, 24 h after, and 1 week after treatment (08:30–09:30 h). Group-housed mice (2–4/cage, 12-hour light/dark cycle, 23 °C) were provided ad libitum access to tap water and standard chow (NIH #31, Teklad) and acclimated to handling and restraint.

Human hormone and cholesterol quantification

For the nine-steroid panel method, 1 ml aliquots of plasma samples were prepared with internal standards for LC-APPI-MS/MS analysis as previously described [16]. Compounds measured in plasma by 6490 Triple Quadrupole mass spectrometer with iFunnel technology (Agilent) operated in positive mode under multiple-reaction monitoring (MRM) conditions included: cortisol, cortisone, 11-deoxycortisol, corticosterone, androstenedione, total testosterone, progesterone, 17-hydroxyprogesterone, dehydroepiandrosterone, and cholesterol [16]. Plasma samples from 2 of the 14 subjects were not evaluated for cholesterol due to insufficient sample volume.

Mouse serum cholesterol quantification

Serum separated by centrifugation (7000 × g, 3 min) using Beckton Dickson vials was stored at –80 °C until analysis. Samples were prepared as previously described by Kelley with slight modifications [17]. Coprostan-3-ol (20 µg, Sigma) added to each 50 µl sample was the surrogate internal standard. Saponified, extracted and dried samples were derivatized with bis-trimethylsilyltrifluoroacetamide plus 1 % trimethylchlorosilane (ThermoFisher) for 1 h (60 °C) and injected onto a Trace 1310GC TSQ 8000 Evo Mass Spectrometer (Thermo Scientific) using a ZB-1701 column (Phenomenex). Retention times were confirmed using standards (Sigma), and the National Institutes of Standards and Technology mass spectral library (data version 14).

Mouse glucocorticoid quantification

For each 50 µl serum sample, an internal standard of cortisol-(9,11,12,12)-D4 (Cerilliant, Round Rock, TX, USA) (10 pmol) was added. Dried samples were dissolved in 25 µl isopropyl alcohol/water (1:3) (v/v) and 10 pmol 17α-hydroxyprogesterone (2,3,4,¹³C₃) (Cerilliant, Round Rock, TX, USA) was added as a recovery standard. Samples were analyzed by LC/MS/MS (Agilent 6495A QQQ). Glucocorticoids were separated by reversed-phase chromatography (Acquity UPLC BEH C18; 2.1 I.D. × 150 mm column). Compounds were ionized using an Agilent Jet-Stream ESI source (gas temp, 250 °C; gas flow, 12 l/min; and capillary voltage, 4000). Data were collected using a multiple reaction monitoring method. For corticosterone the area ratio for T1/T2 was 0.83.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7.03. Data were tested for normality using the Shapiro–Wilke test. Baseline and 24-hour data human data, and mouse

baseline and other time points data were compared using two-tailed paired Student's *t*-tests so that each subject or animal could serve as its own control. Each treated mouse was also compared to the control group. The *p*-value for statistical significance was < 0.05.

Results

Acute statin effects in human participants

►Figure 1a shows that circulating cholesterol was unchanged 24 h after the single 2 mg pitavastatin dose compared to baseline. The mean baseline plasma cholesterol of study participants was 218.8 ± 13.2 mg/dl (reference range: borderline high, > 200 and < 240).

Morning plasma concentrations of aldosterone, corticosterone, cortisone, and 11-deoxycortisol (►Fig. 1c, e, f) were significantly decreased 24 h after pitavastatin treatment by 35 % (*p* = 0.034), 11 % (*p* = 0.048), and 45 % (*p* = 0.039), respectively. Post-treatment morning plasma cortisol tended to be decreased but was not significantly lower (*p* = 0.12). Circulating androgens were not acutely affected (*p* = 0.87).

Glucocorticoids and metabolites measured remained within the range of diurnal steroid reference values derived from healthy adult women and men [18].

Acute statin effects in mice

Circulating cholesterol did not differ between mice treated with atorvastatin or vehicle, nor did post-treatment cholesterol of treated mice differ from their own baseline values. Serum cholesterol at both 24 h and 1-week post-treatment did not differ from baseline (►Fig. 2a).

Three-day atorvastatin treatment (10 mg/kg/d) did not significantly alter circulating corticosterone 24 h or 1 week after the final dose of atorvastatin in comparison to baseline (*p* = 0.56, *p* = 0.87) (►Fig. 2b).

Discussion

This investigation of the acute effects of statins on the steroidogenic pathway identified reduced levels of corticosterone, cortisone, 11-deoxycortisol, and aldosterone after a single dose of pitavastatin. Despite a tendency toward decreased 24-hour cortisol concentrations, cortisol and aldosterone levels did not change significantly.

There are several possible explanations for the observed changes: First, inhibition of HMG-CoA may acutely affect the *de novo* synthesis of cholesterol in adrenocortical cells, which is not detected in serum cholesterol. Under normal conditions, *de novo* cholesterol production is not the main supply of cholesterol for adrenal cells [3]. However, this mechanism is sufficient to support adequate cortisol production in circumstances of deficient circulating cholesterol, such as in cases of abetalipoproteinemia or LDL receptor defects [19, 20]. Alternatively, an independent role of statins on the steroidogenic pathway and the hypothalamic-pituitary-adrenal-(HPA) axis could be hypothesized since statins exert effects beyond cholesterol reduction, including anti-inflammatory, immunomodulatory, and others [21].

The lack of a major effect on cortisol is unsurprising given the data from post-marketing surveillance of statins that revealed no abnormalities of HPA-axis hormones. Furthermore, previous studies failed to identify a consistent and reproducible effect on cortisol production [6, 22–24]. A potential increase in cortisol among individuals taking statins was suggested by a recent meta-analysis [25]; however, clinical significance was limited due to the small number of analyses and widely varied effect size (CI: 1.8–10.8 %). Recently, atorvastatin was shown to decrease adrenal steroid production in H295R cells and in major endocrine tissues of male rats. [26].

Given the principal role of cortisol in homeostasis, the HPA-axis is tightly regulated [27]. Small changes in concentration of the substrate molecules are typically compensated for via upregulation of CRH and/or ACTH production. This may not be the case for aldosterone production, which is mainly under renin-angiotensin control. Small decreases in aldosterone have been described with lipophilic statins use [28].

Even small effects on the steroidogenic pathway support a potential interaction for statins in certain conditions. We recently described that cholesterol supply and use in various adrenal pathologies is distinct [29]. Specifically, individuals with bilateral adrenocortical hyperplasia (BAH) showed a higher degree of de novo production of cholesterol and decreased uptake of circulating cholesterol than cortisol producing adenomas [29]. This finding, and the theoretical role of statins in cell proliferation and apoptosis, and statin influence on angiogenesis and metastases, may suggest a potential benefit from the adjuvant use of statins in these conditions.

Recently, a large study showed that statin users have lower levels of certain androgens [30]. The effect of statins on endogenous sex steroids has been studied in other smaller investigations with inconsistent results [31–36]. There are a few reports on the effects of statin therapy in steroid levels in both men and women, as well [37–39].

Our study has certainly limitations. First, the numbers of animals and human subjects were small. Unfortunately, these studies cannot be repeated in our cohort, and the available sample volume from mice was prohibitive of additional steroid measurements. Second, the suitability of rodents as a model for human lipoprotein metabolism has been debated due to key differences between species. Mice lack cholesteryl ester transfer protein (CETP) and thus carry most plasma cholesterol in HDL versus humans who carry most in LDL [40].

Conclusions

A single dose of pitavastatin acutely decreased the concentrations of certain steroid hormone precursors in our small cohort but did not impair significantly cortisol and aldosterone. The observed mild acute dysregulation may be significant in individuals with an underlying adrenal disease. Further randomized placebo-controlled studies can precisely identify these acute changes.

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Fig. 1.

Acute response of circulating cholesterol and glucocorticoids to single 2 mg dose of pitavastatin. Plasma concentrations of **a** cholesterol, **b** androstenedione, **c** corticosterone, **d** cortisol, **e** cortisone, **f** 11-deoxycortisol, **g** DHEA, and **h** 17-hydroxyprogesterone (17-OHP) at baseline and 24 h after single dose of pitavastatin (2 mg). Data represent mean ± SEM; n = 12–14; * p < 0.05, two-tailed paired Student t-tests.

Fig. 2.

Acute response to 3-day atorvastatin treatment of circulating cholesterol and corticosterone in mice. Serum **a** cholesterol and **b** corticosterone in mice were unchanged in mice 24 h and 1 week after treatment (3 d) with atorvastatin; $n = 7$, group; data represent mean $\pm$ SEM; $p < 0.05$, two-tailed paired Student t -tests.