Abiraterone acetate treatment lowers 11-oxygenated androgens

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Abstract

Context: The human adrenal is the dominant source of androgens in castration-resistant prostate cancer (CRPC) and classic 21-hydroxylase deficiency (21OHD). Abiraterone, derived from the prodrug abiraterone acetate (AA), inhibits the activity of cytochrome P450 17-hydroxylase/17,20-lyase (CYP17A1), the enzyme required for all androgen biosynthesis. AA treatment effectively lowers testosterone and androstenedione in 21OHD and CRPC patients. The 11-oxygenated androgens are major adrenal-derived androgens, yet little is known regarding the effects of AA administration on 11-oxygenated androgens.

Objective: To test the hypothesis that AA therapy decreases 11-oxygenated androgens.

Design: Samples were obtained from 21OHD or CRPC participants in AA or AA plus prednisone (AAP)-treatment studies, respectively.

Methods: We employed liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure the 11-oxygenated androgens, 11 β -hydroxyandrostenedione, 11-ketoandrostenedione, 11 β -hydroxytestosterone, and 11-ketotestosterone, in plasma or serum samples from six 21OHD and six CRPC patients before and after treatment with AA or AAP, respectively.

Results: In CRPC patients, administration of AAP (1000 mg/day AA with prednisone and medical castration) lowered all four 11-oxygenated androgens to below the lower limits of quantitation (<0.1–0.3 nmol/L), equivalent to 64–94% reductions from baseline. In 21OHD patients, administration of AA (100–250 mg/day for 6 days) reduced all 11-oxygenated androgens by on average 56–77% from baseline.

Conclusions: We conclude that AA and AAP therapies markedly reduce the production of the adrenal-derived 11-oxygenated androgens, both in patients with high (21OHD) or normal (CRPC) 11-oxygenated androgens at baseline, respectively. Reduction of 11-oxygenated androgens is an important aspect of AA and AAP pharmacology.

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Introduction

Unlike the adrenals of most small mammals and other vertebrates, human adrenals express the enzyme cytochrome P450 17-hydroxylase/17,20-lyase (CYP17A1) and therefore produce not only cortisol but also 19-carbon steroids (androgens and androgen precursors). The zona reticularis (ZR) maintains high ACTH-dependent

expression of CYP17A1 and the regulatory protein cytochrome *b*₅, which are required to efficiently convert pregnenolone to dehydroepiandrosterone (DHEA) (1). The adrenal exports large amounts of DHEA as the sulfate (DHEAS), and circulating DHEAS concentrations exceed those of all other steroids by up to several

orders of magnitude throughout most of adult life. The low expression of 3β -hydroxysteroid dehydrogenase/isomerase (3β HSD) in the ZR limits conversion of DHEA to active androgens, yet the human adrenal directly produces moderate amounts of androstenedione (A4) and very small amounts of testosterone (T) (2) (Fig. 1).

While the testes are the primary source of the androgen T in normal adult men, the adrenals produce other 19-carbon steroids, and adrenal-derived androgens (active androgens either directly produced from the adrenal or generated in peripheral tissues from precursors synthesized in the adrenals) are a significant component in many scenarios. In prepubertal children, the expansion of the ZR and rise of adrenal-derived androgens manifest as the development of axillary and pubic hair during the process of adrenarche (3). Genetic defects in cortisol biosynthesis, collectively known as congenital adrenal hyperplasia (CAH), feature androgen excess in most

forms, including 21-hydroxylase deficiency (21OHD, the most common), 11β -hydroxylase deficiency, and in girls also 3β HSD type 2 deficiency (4). In addition, the standard initial treatment for men with metastatic prostate cancer is androgen deprivation therapy (ADT) with medical castration (5). While ADT eliminates testicular T production, adrenal-derived androgens persist and precipitate treatment failure upon transition to castration-resistant prostate cancer (CRPC) (6), which has been difficult to treat and is often fatal.

In both 21OHD and CRPC, strategies to lower adrenal-derived androgens have been developed. In 21OHD, supraphysiologic glucocorticoid dosing has been a successful strategy to lower ACTH and adrenal 19-carbon steroid production from high to normal (7). In CRPC, reduction or blockade of adrenal-derived androgens has become an effective treatment strategy, but the degree of suppression or antagonism must be essentially complete.

Figure 1

Abbreviated pathways of adrenal steroid biosynthesis. DHEA is primarily sulfated by sulfotransferase 2A1 (SULT2A1) to DHEAS, but a fraction is converted to A4. A4 is a good substrate for CYP11B1, yielding 11OHA4, and 11OHA4 is metabolized primarily in peripheral tissues to 11KT. Abbreviations not in text: 11β HSD2, 11β -hydroxysteroid dehydrogenase type 2; AKR1C3, aldo-keto reductase 1C3 (17β HSD5); Cyt b_5 , cytochrome b_5 ; StAR, steroidogenic acute regulatory protein.

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Besides third-generation anti-androgens, CYP17A1 inhibitors have become a mainstay of CRPC treatment (8). Abiraterone acetate (AA), the prodrug of abiraterone, in combination with prednisone has been shown to improve survival in CRPC patients (9, 10) as well as when added to ADT prior to the development of CRPC (11, 12). Using highly sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays, 1000 mg/day AA plus prednisone 5 mg twice daily (AAP therapy) lowers the traces of circulating T in most men with CRPC from ~0.3 nmol/L to <0.01 nmol/L (13). In 2014, we repurposed AA for the treatment of classic 21OHD in combination with physiologic hydrocortisone. The six enrolled participants received 100 or 250 mg of AA each morning plus 20 mg/day hydrocortisone and fludrocortisone acetate for 6 days. Using LC-MS/MS, the study found that AA at 250 mg/day normalized A4 and T on day 7 in all participants (14).

Beyond the traditional 19-carbon steroids DHEA, A4, and T, the human adrenal produces substantial amounts of 11-oxygenated androgens (Fig. 1). Of these, the major direct adrenal product is 11 β -hydroxyandrostenedione (11OHA4) (2) via 11 β -hydroxylation of A4, and 11-ketotestosterone (11KT) is the major active androgen derived from 11OHA4 through peripheral metabolism (15). Although 11KT has been long recognized as the primary active androgen in bony fishes and 11OHA4 was identified in human serum decades ago, a broader significance of 11-oxygenated androgens in human beings has only recently emerged (16). We have shown that 11KT is the dominant circulating androgen during normal and premature adrenarche (15), in women and men with poorly controlled 21OHD (17), and in postmenopausal women (18). Other studies suggest that circulating 11KT concentrations exceed those of T in most women, particularly those with polycystic ovary syndrome (19), and that 11-oxygenated androgens contribute to the progression of CRPC (20). While AA has been shown to lower A4 and T in both 21OHD (from high to normal) (14) and CRPC (from normal or low to undetectable) (13), the changes in 11-oxygenated androgens were not previously assessed in AA studies. The purpose of this study is to determine the effects of AA administration on the 11-oxygenated androgens 11OHA4, 11KT, as well as 11-ketoandrostenedione (11KA4) and 11 β -hydroxytestosterone (11OHT), to broaden our understanding of AA pharmacology.

Methods

AA was administered to the 21OHD patients after the first-morning dose of hydrocortisone on days 1

through 6, and plasma samples from were collected at 0800 h prior to all medications on days 1, 6, 7, and 8 (NCT01495910). Baseline samples reflected the effect of hydrocortisone 20 mg/day in 2–3 divided doses prior to two dose-escalated AA treatment periods, 100 and 250 mg/day, after which the primary endpoint was met (14). The serum A4 at baseline exceeded 1.5 times the upper limit of normal in all participants (>12 nmol/L). Serum samples were collected from six CRPC patients who received AAP treatment between 09/03/2015 and 03/23/2017. Patients with metastatic CRPC were recruited through the Department of Hematology and Oncology, Taussig Cancer Institute at the Cleveland Clinic. The protocol (The Origins of Resistance to Hormonal Therapy in Advanced Prostate Cancer, case 7813) was approved by the institutional review board, and all subjects provided written informed consent. To qualify, subjects had not received prior ketoconazole treatment or chemotherapy or were currently treated with 5 α -reductase inhibitors. Serum samples were collected and banked for research purposes during standard-of-care treatment with AAP, and all six available pairs of pre- and post-AAP samples were used. Two blood samples were collected from each patient – prior to starting AAP treatment and following at least 4 weeks of treatment between 2 and 15 h after the 1000 mg daily dose of AA. Samples were collected in Vacutainer Plus serum blood collection tubes (#BD367814, Becton Dickinson, Franklin Lakes, NJ) and allowed to clot. Tubes were centrifuged at 1430 *g* for 10 min. Serum aliquots were frozen at –80°C until processing. The studies were approved by the institutional review boards of the University of Michigan or Cleveland Clinic, and all participants gave written informed consent.

Serum or plasma (0.1 mL) was combined with internal standards (Table 1), diluted with water, and loaded onto a supported liquid extraction cartridge (Isolute, Biotage, Charlotte, NC). Steroids were eluted twice with 0.7 mL methyl-*tert*-butyl ether, dried under vacuum (Savant, Thermo Fisher), and reconstituted in 0.1 mL 40:60 methanol:water. LC-MS/MS was performed as previously described (18), with the following modifications. Steroids were quantified with an Agilent 6495 triple quadrupole mass spectrometer and Agilent 1260 and 1290 dual front-end HPLC/UPLC equipped with a 3 $\times$ 10 mm 5- μ m particle size C₄ HypersilGOLD cartridge (Thermo Fisher) and a Kinetex 2.1 $\times$ 150 mm 2.6- μ m particle size biphenyl resolving column (Phenomenex, Torrance, CA) in electrospray ionization mode. Mobile phases consisted of 0.2 mmol/L aqueous ammonium fluoride (phase A) and methanol with 0.2 mmol/L ammonium fluoride (phase B).

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Table 1 List of steroid analytes, standards, internal standards, and mass spectrometry parameters.

Steroid	Source	MRM (m/z)	Qual (m/z)	RT (min)	LLOQ (nmol/L)
Progesterone	Cerilliant	315.2/97.0	109.1	14.4	0.09
Progesterone-d9	C/D/N Isotopes	324.2/97.0	113.1	14.4	
17-Hydroxyprogesterone	Cerilliant	331.2/97.0	313.0	11.4	0.1
17-Hydroxyprogesterone-d8	C/D/N Isotopes	339.2/100.0	113.0	11.3	
Corticosterone	Cerilliant	347.2/121.1	329.2	10.6	0.6
Corticosterone-d8	C/D/N Isotopes	355.2/337.1	125.1	10.5	
11-Deoxycorticosterone	Cerilliant	331.2/97.0	109.0	13.0	0.06
11-Deoxycorticosterone-d8	Sigma-Aldrich	339.2/100.0	113.0	13.0	
11-Deoxycortisol	Cerilliant	347.2/97.0	109.0	10.0	0.01
11-Deoxycortisol-d5	Sigma-Aldrich	352.2/100.1	113.1	9.9	
Cortisol	Cerilliant	363.2/121.1	327.3	7.3	0.6
Cortisol-d4	Cerilliant	367.2/121.1	97.0	7.3	
Cortisone	Cerilliant	361.2/163.0	121.1	8.0	0.7
Cortisone-d8	Cerilliant	369.0/169.0	120.0	7.9	
Androstenedione	Cerilliant	287.0/97.0	109.0	13.0	0.03
Androstenedione-d7	Cambridge Isotopes	294.0/100.0	113.0	13.0	
Testosterone	Cerilliant	289.2/97.0	109.0	11.3	0.02
Testosterone-d3	C/D/N Isotopes	292.0/97.0	109.0	11.3	
11-Hydroxyandrostenedione	Steraloids	303.2/120.9	285.1	10.5	0.3
11-Hydroxyandrostenedione-d3	RTI	306.2/288.2	122.1	10.4	
11-Ketoandrostenedione	Steraloids	301.2/257.0	120.9	10.7	0.1 or 265.1
11-Hydroxyandrostenedione-d3	RTI	306.2/288.2	122.1	10.4	
11-Hydroxytestosterone	Steraloids	305.3/269.0	105.0	10.5	0.1
11-Ketotestosterone-d3	RTI	308.3/262.3	123.1	9.3	
11-Ketotestosterone	Steraloids	303.2/120.9	259.1	9.3	0.1
11-Ketotestosterone-d3	RTI	308.3/262.3	123.1	9.3	

LLOQ, lower limit of quantitation (extrapolated signal-to-noise ratio of 3); m/z, mass to charge ratio; MRM, precursor and product ions for mass ratio monitoring; Qual, qualifier ion; RT, retention time; RTI, The National Heart, Lung and Blood Institute RTI International metabolite Standards Synthesis Center.

Samples were first loaded onto the C₄ cartridge with isocratic 35% mobile phase B at a flow of 0.2 mL/min using the 1260 HPLC pumps, and the early eluting polar matrix elements were routed to waste. Immediately prior to the elution of the first compound of interest from the C₄ cartridge, a 10-port valve routed the flow from the 1290 UPLC pumps through the C₄ cartridge to the biphenyl column in serial configuration, and mobile phase B was stepped to 55% to expedite elution of steroids from the C₄ cartridge to the biphenyl column. After the last compound of interest was eluted from the C₄ cartridge, the 10-port valve routed the flow from the 1260 HPLC pumps through the C₄ cartridge to waste for washing, which eliminated many non-polar interfering matrix elements and prepared the cartridge for the next injection. Simultaneously, the 10-port valve directed the flow from the 1290 UPLC pumps directly through the biphenyl column first to waste and then from 3.01 to 14.9 min into the source of the Agilent 6495 triple quadrupole tandem mass spectrometer using a methanol/water gradient (Table 2). The steroids were quantitated using dynamic multiple reaction monitoring (DMRM) mode (Table 1).

The lower limit of quantitation (LLOQ, Table 1) for each analyte was estimated from the signal-to-noise ratio of pooled samples, using the Mass Hunter 'peak to peak' method in a noise region window lacking interfering peaks of 0.25 min most often post-peak, and extrapolated to a concentration that yields a signal-to-noise ratio of 3. Review of extracted ion chromatograms confirmed that all samples with values reported above the LLOQ gave reliably quantifiable peaks at the correct retention time. Baseline versus AA or AAP values were compared with two-tailed paired Wilcoxon signed rank tests setting $\alpha=0.05$.

Results

CRPC patients

For the six CRPC patients, baseline 11OHA4 concentrations ranged 2–5 nmol/L and 11KT 0.4–1.4 nmol/L, with 11OHT and 11KA4 values lower. After treatment with 1000 mg AA daily plus prednisone 5 mg twice daily, the active androgens 11OHT and 11KT (15) were both suppressed to below the LLOQ (0.1 nmol/L), a 73–93% reduction from baseline ($P=0.03$, Fig. 2). Similarly, 11KA4 and

Table 2 Chromatography scheme for LC-MS/MS.

Time (min)	% A	% B	Routing	Flow (mL/min)
1260 HPLC Pump 1				
0.00	65	35	1→Waste	0.20
0.70	65	35	1→Waste	0.20
0.71	65	35	Waste	0.20
0.80	65	35	Waste	0.20
0.85	0	100	Waste	0.20
1.20	0	100	Waste	0.50
2.50	0	100	Waste	0.50
2.51	0	100	Waste	0.10
3.01	0	100	1→Waste	0.10
3.90	0	100	1→Waste	0.10
3.91	0	100	1→Waste	0.10
7.40	0	100	1→Waste	0.10
10.00	0	100	1→Waste	0.20
10.01	65	35	1→Waste	0.50
12.00	65	35	1→Waste	0.50
12.10	65	35	1→Waste	0.20
1290 UPLC Pump 2				
0.00	65	35	2→Waste	0.20
0.70	65	35	2→Waste	0.20
0.71	65	35	1→2→Waste	0.20
0.80	45	55	1→2→Waste	0.20
3.01	45	55	2→MS	0.20
12.50	6.6	93.4	2→MS	0.20
12.51	5	95	2→MS	0.20
14.90	0	100	2→MS	0.25
15.00	0	100	2→Waste	0.25
15.20	0	100	2→Waste	0.50
16.20	0	100	2→Waste	0.50
16.21	65	35	2→Waste	0.40
19.21	65	35	2→Waste	0.40

%A, percent mobile phase A; %B, percent mobile phase B; 1, column 1 = C₄ cartridge; 2, column 2 = biphenyl resolving column; arrows indicate routing of flow.

11OHA4 decreased to below LLOQ (0.1 or 0.3 nmol/L, respectively) in all patients, which are 64–94% reductions from baseline ($P=0.03$). For comparison, AAP therapy also lowered A4 69–90% from baseline and T to below LLOQ (0.02 nmol/L) in all patients ($P=0.03$, Fig. 2). These data indicate that AAP therapy markedly lowers 11-oxygenated androgens in CRPC patients.

21OHD patients

At baseline prior to the first treatment period, the six 21OHD patients had baseline A4 and T values well above the normal range for women. We confirmed that, as we showed previously (14), AA treatment reduced A4 and T into the normal range by day 6–7 in half or all women at 100 mg/day or 250 mg/day, respectively ($P=0.03$, Fig. 3). Similarly, the 11-oxygenated androgens fell markedly, particularly in those with the highest baseline values, and did not return to baseline by day 8. With 250 mg/day AA, 11OHA decreased

Figure 2

Reductions in conventional and 11-oxygenated androgens in CRPC patients with AAP treatment. Symbols show individual patient data from baseline (BL) and after at least 4 weeks of AAP therapy. The dotted lines indicate LLOQ for each steroid, and values <LLOQ are plotted midway between 0 and LLOQ. Conversion factors from nmol/L to ng/dL: multiply value by 28.64 for A4, 28.84 for T, 30.24 for 11OHA4 and 11KT, 30.04 for 11KA4, and 30.44 for 11OHT.

64–98%, 11KA decreased 63–98%, 11OHT decreased 51–90%, and 11KT decreased 55–97% ($P=0.03$, Fig. 3).

Progesterone and 11-deoxycorticosterone changes

Because administration of AA inhibits both 17-hydroxylase and 17,20-lyase activities of CYP17A1, 17-deoxysteroids upstream of androgens rise, and glucocorticoid administration attenuates this rise (21, 22). In patients with 21OHD, the cytochromes P450 CYP3A4

Figure 3

Changes in conventional and 11-oxygenated androgens with AA in patients with 21OHD. Symbols show individual patient data from baseline (day 1) and days 6–8 for 6 days of AA therapy at 100 mg/day (Period 1) or 250 mg/day (Period 2). Dotted lines indicate upper limit of normal ranges for premenopausal women (18). Conversion factors from nmol/L to ng/dL: multiply value by 28.64 for A4, 28.84 for T, 30.24 for 11OHA4 and 11KT, 30.04 for 11KA4, and 30.44 for 11OHT.

and CYP2C19 in the liver 21-hydroxylate circulating progesterone to 11-deoxycorticosterone (DOC) (23). We therefore also sought to assess changes in progesterone and DOC in patients with 21OHD during AA treatment.

In the 21OHD patients, median progesterone increased as anticipated by 148% in period 1 and by 114% in period 2 on day 6; however, proportionate increases in DOC did not accompany these increases in progesterone. In period 1, median DOC rose only 3%, and in period 2, DOC did not change. Consequently, the median progesterone/DOC ratio increased by 121% in period 1 and by 106% in period 2 ($P=0.03$, Fig. 4).

Figure 4

Changes in progesterone, DOC, and progesterone/DOC (Prog/DOC) ratio in 21OHD patients with AA treatment. Symbols show individual patient data from baseline and after 6 days of AA therapy at 100 mg/day (Period 1) or 250 mg/day (Period 2). Conversion factors for ng/dL: multiply value by 31.45 for progesterone and 33.05 for DOC.

Figure 5

Changes in adrenal 21-carbon steroids from CRPC patients at baseline and with AAP treatment. Symbols show individual patient data from baseline and after at least 4 weeks of AAP therapy. Conversion factors for ng/dL: multiply by 36.25 for cortisol, 34.65 for corticosterone, 31.45 for progesterone, and 33.05 for DOC.

Unlike the 21OHD patients, the CRPC patients experienced a 20-fold rise in median DOC (0.11 to 2.25 nmol/L). This rise in DOC was accompanied by substantial rises in median corticosterone (2.25 to 195.6 nmol/L) and progesterone (0.13 to 5.33 nmol/L) ($P=0.03$ for all changes, Fig. 5). Median cortisol decreased by 81% from baseline, yet this adrenal axis suppression was variable across the six patients. In patients 4, 5, and 6, on-treatment cortisol ranged 62.6–82.6 nmol/L, while in patients 1, 2, and 3, treatment cortisol ranged 1.0–16.1 nmol/L (Fig. 5).

Discussion

The major finding of this study is that AA and AAP therapies effectively and consistently lower 11-oxygenated androgens in both 21OHD and CRPC patients, respectively, in parallel with conventional androgens A4 and T. AA or AAP therapies were effective in lowering 11-oxygenated androgens from high values (up to ten times the upper limit of normal) to within or much closer to normal in 21OHD patients and from normal values to below LLOQ in CRPC patients, illustrating efficacy in two very different conditions. Although the cohorts consisted of only six patients each, the results were consistent and dramatic across all patients in both cohorts, and the results were replicated in a second treatment period for the 21OHD patients. Consequently, we report these results with confidence.

Our data support the hypothesis that AA therapy lowers 11-oxygenated androgens. The 11-oxygenated androgens are now recognized as the major androgens in patients with 21OHD (17), and new strategies to lower

A4 and T (14, 24, 25, 26) must also reduce 11-oxygenated androgens, as we now show for AA. As observed for A4 and T in the 21OHD study (14), 11-oxygenated androgens remained below baseline on day 8, 48 h after the last AA dose, consistent with extremely tight binding of abiraterone to CYP17A1 (27). Although 11-oxygenated androgens were previously neglected in CRPC, prostate cancer cell lines contain enzymes to convert 11OHA4 to active androgens (28, 29, 30), and 11-oxygenated androgens promote growth of these cells (20). Our data suggest that inhibition of 11-oxygenated androgen production might significantly contribute to the efficacy of AAP in the treatment of CRPC, and direct inhibition of adrenal CYP17A1 and 11-oxygenated androgen synthesis, which is not achieved with ADT alone, might contribute to the efficacy of AAP as initial treatment of metastatic prostate cancer.

Complete or partial aldosterone deficiency contributes to the propensity for infants and children with 21OHD to experience adrenal crises, yet hypertension is observed in some adults with 21OHD (7). One potential mechanism of this phenomenon is the extra-adrenal 21-hydroxylation of circulating adrenal-derived progesterone to DOC via hepatic P450 enzymes (23), as is observed in normal pregnancy with placenta-derived progesterone (31). In one study of patients with classic 21OHD, DOC did not rise with cosyntropin stimulation (32), consistent with extra-adrenal genesis. Alternatively, human CYP17A1 has a small amount of progesterone 21-hydroxylase activity, which is accentuated with (16 α ,17- 2 H $_2$)-progesterone substrate, a consequence of metabolic switching due to intramolecular kinetic isotope effects (33). If DOC production in 21OHD was solely due to hepatic metabolism of circulating progesterone, the AA therapy, which raises

progesterone, should also raise DOC proportionately, but if CYP17A1-catalyzed the DOC formation, AA should lower DOC production. Our observation that DOC remains relatively stable with AA treatment for 21OHD despite a >2-fold increase in progesterone suggests that a portion of DOC derives from both pathways.

We conclude that AA decreases adrenal-derived 11-oxygenated androgens in patients with either very low or very high androgens at baseline. This action of AA is likely to contribute significantly to the efficacy of AAP in the treatment of patients with CRPC and of AA in the treatment of patients with 21OHD.

Declaration of interest

R J A is a consultant for Janssen Pharmaceuticals. The other authors have nothing to disclose.

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