 Prague Medical Report / Vol. 110 (2009) No. 3, p. 222–230

Finasteride Treatment and Neuroactive Steroid Formation

Dušková M.[1], Hill M.[1], Hanuš M.[2], Matoušková M.[2], Stárka L.[1]
[1]Institute of Endocrinology, Prague, Czech Republic;
[2]Urocenrum, Prague, Czech Republic

Received January 9, 2009; Accepted April 30, 2009.

Key words: 5α-reductase – Finasteride – Neuroactive steroids – Benign prostatic hyperplasia – Depression – Anxiety

Abstract: Finasteride is the 5α-reductase inhibitor that received clinical approval for the treatment of human benign prostate hyperplasia and androgenetic alopecia. The 5α-reductase is enzyme responsible for the reduction of testosterone to dihydrostestosterone, progesterone to dihydroprogesterone and deoxycorticosterone to dihydrodeoxycorticosterone, steroids modulating the action of γ-aminobutyric acid on GABA receptors. These neuroactive steroids possess anticonvulsant, antidepressant and anxiolytic effects. The objective of the study was to determine the effect of finasteride therapy on a broad steroid spectrum in men with benign prostate hyperplasia. A group of 20 men with benign prostate hyperplasia was involved in the present study. Finasteride in the daily dose of 5 mg/day was administrated for 4 months. In all individuals, their hormonal profile of steroid hormones was determined before and after 4 months lasting finasteride treatment. Finasteride treatment resulted in a significant decrease all α-reduced and increase of most 5β-reduced metabolites of testosterone and progesterone as well as in an increase of 7α-hydoxyderivatives, which are known as neuroactive steroids acting by modulation of $GABA_A$ and NMAD receptors in the brain. In the course of finasteride treatment the decrease of the concentration of circulating steroids with known inhibitory activity on GABA-ergic excitation in the brain is very probably an important factors contributing to the development of the symptoms of depression seen in some isolated cases of finasteride administration.

This study was supported by grant No. NR/8525 – 5 of the Internal Grant Agency of the Ministry of Health of the Czech Republic.

Mailing Address: Prof. Luboslav Stárka, MD., RNDr., DSc., Steroid Hormone Department, Institute of Endocrinology, Národní 8, 11694 Prague 1, Czech Republic; Phone: +420 224 905 344; Fax: +420 224 905 325; e-mail: lstarka@endo.cz

Introduction

Finasteride, a 5α-reductase inhibitor, is widely used for amelioration of symptoms of benign prostate hyperplasia or to stop hair loss in androgenetic alopecia as a drug reducing the formation of dihydrotestosterone from testosterone and thus decreasing the growth of prostate or the hair loss. Several clinical observation [1–5] and experiments on laboratory rodents have demonstrated that finasteride treatment [6, 7, 8, 9, 5, 10] can possible give rise to depression and mood modification.

These finding suggest that finasteride might induce depressive symptoms. Therefore this medication should be prescribed cautiously for patients with high risk of depression [3]. Depression associated to marked anxiety in some cases promptly resolved after suspension of the drug in men with androgenetic alopecia [4].

These effects of finasteride could be ascribed to its activity in blocking steroid 5α-reductase, which blocked not only dihydrotestosterone formation in the periphery, but also changed the whole pattern of androstane and pregnane derived neuroactive steroids, acting on GABA receptors.

The aim of this study was to learn the changes in the broad spectrum of neuroactive steroids after finasteride treatment in dose of 5 mg/day.

Subjects and Methods

Patients

We examined the groups of 20 men in the average age of 69.2 ± 6.5 years with benign prostate hyperplasia, who searched medical help for this diagnosis and had no treatment for it before. Male subjects over the age of 50 years were recruited from urology outpatients with obstruction or irritation symptoms of benign prostate hyperplasia verified by ultrasonography. Subjects with other significant urological diseases such as other obstructive uropathy, malignancy, haematuria or apparent non-urological diseases were excluded as well as patients receiving any drugs of hormonal or plant origin for BPH treatment. Finasteride (Penester, Zentiva) in the daily dose of 5 mg was administrated for 4 months. The steroids spectrum was determined before starting the treatment and at the end of 4 months finasteride treatment. Local Ethical Committee approved the study and all patients signed informed consent form before taking part in the study.

Sample collection

Blood was withdrawn from cubital vein in morning hours between 7–10 h a.m. Cooled plastic tubes were used for blood sampling. The plasma was obtained after centrifugation for 5 minutes at 2000 g at 0 °C. The plasma samples were stored at –20 °C until analyzed.

Steroids and chemicals

The steroids were from Steraloids (Wilton, NH, USA). The solvents for the extraction and HPLC, were of an analytical grade, from Merck (Darmstadt,

Germany). The derivatization agent Sylon BFT was purchased from Supelco (Bellefonte, PA, USA).

Instruments

The GC-MS system was supplied by Shimadzu (Kyoto, Japan). The GCMS-QP2010 Plus system consisted of a gas chromatograph equipped with automatic flow control, AOC-20s autosampler and a quadrupole electron-impact detector with an adjustable electron voltage of 10–195 V. A capillary column with a medium polarity RESTEK Rxi (diameter 0.25 mm, length 15 m, film thickness 0.1 μm) was used for analyses.

Steroid analysis

The levels of steroids listed in Table 1 were measured in plasma using GC-MS. The unconjugated steroids were extracted from 1 ml of plasma with diethyl-ether (3 ml). The diethyl-ether extract was dried in the block heater at 37 °C. The lipids in the dry residue of the diethyl-ether extract were separated by partitioning between a mixture of methanol-water 4:1 (1 ml) and pentane (1 ml). The pentane phase was discarded and the polar phase was dried in the vacuum centrifuge at 60 °C (2 h). The dry residue from the polar phase was derivatized first with methoxylamine-hydrochloride solution in pyridine (2%) on oxo-groups (60 °C, 1 h). The mixture after the first derivatization was dried in the flow of nitrogen and the dry residue was treated with the reagent Sylon B (99% of bis(trimethylsilyl)-trifluoroacetamide and 1% of trimethylchlorosilane) forming trimethylsilyl derivatives on hydroxy-groups (TMS-MOX derivatives) (90 °C, 1 h). Finally, the mixture after the second derivatization step was dried in the flow of nitrogen, the dry residue was dissolved in 20 μl of isooctane and 1 μl of the solution was used for GC-MS analysis.

Table 1 – Steroids determined in plasma of men treated with finasteride for BPH

Nomenclature according to IUPAC	Trivial name
4-androstene-3,17-dione	androstenedione
3α-hydrox-5α-androstan-17-one	androsterone
3α-hydrox-5β-androstan-17-one	etiocholanolone
3β-hydrox-5α-androstan-17-one	epiandrosterone
3β-hydrox-5β-androstan-17-one	epietiocholanolone
3β-hydroxy-5-androsten-17-one	dehydroepiandrosterone (DHEA)
3β,16α-dihydroxy-5-androsten-17-one	16α-hydroxy-DHEA
3β,7α-dihydroxy-5-androsten-17-one	7α-hydroxy-DHEA
17β-hydroxy-5α-androstan-3-one	5α-dihydrotestosterone
3α-hydroxy-5α-pregnan-20-one	allopregnanolone
3α-hydroxy-5β-pregnan-20-one	pregnanolone
3β-hydroxy-5-pregnen-20-one	pregnenolone
17β-hydroxy-4-androsten-3-one	testosterone

Prior to further processing, the original samples were spiked with 17α-estradiol (as an internal standard) to attain a concentration of 1 ng/ml and 10 ng/ml, respectively. The internal standard was recorded at effective masses m/z = 285 and 416. The addition of internal standard to body fluid before sample preparation assured that the losses during the sample processing were not critical for steroid quantification.

Instrument setup

Electron-impact ionization was used for the analyses. Electron voltage was set up to 70 V and emission current to 160 μA. The temperature of the ion source and interface were maintained at 260 °C and 310 °C, respectively. Analyses were carried out with a constant linear velocity of the carrier gas (He), which was maintained at 60 cm/s. The septum purge flow was set up to 3 ml/min. Samples were injected using the on-column injection mode. The detector voltage was set to 1.4 kV.

Temperature and pressure gradients for the GC-MS analysis of steroids after derivatization and the retention times of the steroids

To effectively utilize the biological material, the individual samples were applied in three independent courses, in each case employing a part of the steroids under investigation. The choices of the steroids measured within the individual courses, the temperature and pressure gradients, and the effective masses used for the measurement in selected ion monitoring (SIM) mode were all optimized to attain minimum limit of detection (LOD) at sufficient selectivity. The temperatures and pressure gradients for the detection of steroids are shown in Table 2. The effective masses, retention times of chromatographic peaks, sequence number of injection for steroid groups and gradients that were used for quantification of individual steroids are shown in Table 3. In all cases,

Table 2 – Temperature and pressure gradients used for steroid quantification

Gradient 1			Gradient 2			Gradient 3		
Injection temperature: 220 °C			Injection temperature: 240 °C			Injection temperature: 220 °C		
Rate [°C/min]	Final temp. [°C]	Hold time [min]	Rate [°C/min]	Final temp. [°C]	Hold time [min]	Rate [°C/min]	Final temp. [°C]	Hold time [min]
–	80	1	–	80	0	–	80	1
40	190	0	40	190	0	40	200	0
4	210	0	2,5	215	0	8	240	0
20	300	5	40	300	8	40	300	8

Injection mode: on column injection, Initial pressure: 34 kPa, Linear velocity: 60 cm/s, Purge flow: 3 mL/min, Ion sourcde temperature: 260°C, Interface temperature: 310°C, Detector voltage: 1.4 kV

the mixtures of authentic standards were processed in the same way as samples. The mixtures were specific for each of the independent courses as mentioned above. The standards were injected in three different amounts for each steroid (10, 100 and 1000 pg).

For evaluation of linearity, increasing volumes of the mixtures of pooled plasma with water for chromatography (300+700, 400+600, 500+500, 600+400, 700+300, 800+200, 900+100 and 1000+0 ml) were assayed. The two-parameter linear regression was used for evaluation of the relationships between peak areas and volume of the plasma.

Statistical data analysis

Wilcoxon's robust paired test was used for evaluation of the effect of finasteride treatment.

Results

The identification of the steroids was based on their chromatographic and mass-spectra characteristics as shown in Table 2.

Table 3 – Analytical characteristics of the steroids

	Steroid	Gradient No.	Peak No.	Effective mass [m/z]	Retention time [min]	σ [min]	Peak width [min]
–	Epiestradiol (internal standard)	1	1	**285**, 416	10,19	0,017	0,07
1	Epietiocholanolone	1	1	**270**, 360	9,65	0,019	0,07
2	Androsterone	1	1	**270**, 360	9,77	0,018	0,07
3	Etiocholanolone	1	1	**270**, 360	9,88	0,018	0,07
4	Epiandrosterone	1	1	270, **360**	10,46	0,016	0,06
5	5α-Dihydrotestosterone	1	1	270, **360**, 391	10,63	0,023	0,09
6	Dehydroepiandrosterone	1	1	**268**, 358	10,47	0,016	0,06
7	Testosterone	1	1	268, 358, **389**	10,86	0,015	0,06
			2	268, 358, 389	11,01	0,014	0,05
8	Pregnenolone	1	1	386, **402**	11,35	0,014	0,06
9	Androstenedione	1	1	313, 344	11,68	0,012	0,05
			2	**313**, 344	11,81	0,012	0,05
–	Epiestradiol (internal standard)	2	1	285, 416	11,3	0,040	0,16
10	Allopregnanolone	2	1	298, **388**	13,2	0,027	0,11
11	Pregnanolone	2	1	298, **388**	13,3	0,021	0,09
–	Epiestradiol (internal standard)	3	1	285, **416**	8,26	0,020	0,08
12	7α-Hydroxy-dehydroepiandrosterone	3	1	266, **387**	8,26	0,020	0,08
13	16α-Hydroxy-dehydroepiandrosterone	3	1	266, 356, **446**	8,86	0,023	0,09
			2	266, 356, 446	8,99	0,027	0,11

Table 4 – Concentration of steroids before and after treatment with 5 mg/day finasteride for 4 months

	I				II				Δ (II–I, %)					
Steroid	n	median	lower quartile	upper quartile	n	median	lower quartile	upper quartile	n	median	lower quartile	upper quartile	Effect of finasteride, p-value	
Androstenedione	20	4.31	3.53	5.82	20	4.18	3.12	7.38	20	–9.3	–36.1	49.4	0.8960	NS
Androsterone	20	0.584	0.439	0.720	20	0.303	0.215	0.383	20	–34.6	–63.6	–14.4	0.0076	*
Etiocholanolone	20	0.077	0.010	0.136	20	0.107	0.015	0.205	20	41.1	–17.4	200.5	0.1403	NS
Epiandrosterone	20	0.508	0.328	0.669	20	0.243	0.176	0.367	20	–36.6	–64.5	–9.3	0.0105	*
Epietiocholanolone	20	0.0042	0.0020	0.0069	20	0.0135	0.0095	0.0554	20	610.3	161.6	1106.1	0.0016	**
Dehydroepiandro-sterone	20	6.25	5.42	9.25	20	4.45	3.44	7.15	20	–19.5	–38.6	9.2	0.0318	*
16α-Hydroxy-dehydroepiandro-sterone	20	0.146	0.096	0.210	20	0.079	0.036	0.158	20	–45.4	–87.7	27.1	0.0419	*
7α-Hydroxy-dehydroepiandro-sterone	20	0.796	0.628	1.144	19	0.464	0.232	0.539	19	–32.4	–66.2	–17.4	0.0035	*
5α-Dihydrotesto-sterone	20	1.048	0.570	2.053	20	0.121	0.078	0.281	20	–76.3	–93.2	–43.6	0.0016	*
Allopregnanolone	20	0.159	0.066	0.280	20	0.042	0.027	0.101	20	–303.1	–13.8	–876.5	0.0048	*
Pregnanolone	20	0.255	0.099	1.153	20	0.202	0.117	0.280	20	–23.3	–92.9	36.4	0.1305	NS
Pregnenolone	20	1.33	0.99	1.67	20	1.13	0.53	1.38	20	–15.2	–49.5	23.3	0.1978	NS
Testosterone	20	15.7	10.6	17.8	19	18.1	15.3	20.2	19	9.2	–4.7	23.9	0.0510	NS

The selectivity of the method was sufficient for all of the steroids as demonstrated in Table 3.

The correct identification of the substances was ensured by congruence of fragmentation pattern and retention time with the standard (at least 2 fragments + retention time(s)). In addition, the correlations were checked between precursors and products considering the known steroid metabolic pathways (data not shown). Further, the steroids containing oxo-group mostly produced two-peak response, thus in this case we also checked the ratios of peak 1 to peak 2 for individual fragments. The LOD was sufficient for all the steroids under investigation ranging from low femtogram to low picogram levels, which depended primarily on the steroid fragmentation pattern (data not shown).

Their concentration in plasma of older men before and at the end of 4 months per oral treatment with 5 mg finasteride per day is given in Table 4. A significant (at the level $p<0.05$) decrease was seen in most of the 5α-reduced metabolites (5α-dihydrotestosterone, androsterone, epiandrosterone, allopregnanolone) and in most of the 5-ene steroids and their 7α- and 16α-hydroxy-derivatives (dehydroepiandrosterone, 7α-hydroxy-dehydroepiandrosterone, 16α-hydroxy-dehydroepiandrosterone), whereas significant increase occurred in one of the 5β-reduced metabolites, epietiocholanolone.

Discussion

Certain medications may contribute to the aetiology of depressive symptoms and disorders; however, drug-induced depression appears to differ symptomatically from classical major depression [11]. Evidence was found linking corticosteroids, interferon-α, interleukin-2, gonadoliberin agonists, angiotensin converting enzyme inhibitors or progestin-releasing implanted contraceptives to the aetiology of atypical depressive syndromes. To these depression-inducing agents influencing endocrine homeostasis finasteride could be counted [12, 4, 3]. Finasteride is a potent 5α-reductase inhibitor approved by Food and Drug Administration for treatment of benign prostate hyperplasia in 1992 and in 1997 for treatment of androgenetic alopecia in men. Finasteride may hold promise also for other dihydrotestosterone-mediated disorders such as acne, facial hirsutism, prostate cancer and symptoms of premature puberty or enormous sexual appetite.

Animal studies suggested that finasteride could alter 5α-reductase activity in some regions of the brain and lead to behavioural and mood changes [13, 14, 15, 10]. One of the well-known mechanisms is the non-genomic effect of several steroids on $GABA_A$ and NMAD receptors modulating the action of excitatory acids (γ-aminobutyric acid or glutamate). In this respect, the action of allopregnanolone and in animals also of tetrahydrodeoxycorticosterone was studied intensively. Less attention was paid to the action on neural system of other steroid hormones and their metabolites such as dihydrotestosterone, 5α-androstane-3α,17β-diol [16], pregnenolone or dehydroepiandrosterone.

In our study we measured some potential neuroactive steroids in plasma. In recent study [17] it was found that plasma concentrations of free, non-conjugated neuroactive steroids pregnenolone and dehydroepiandrosterone correlate with those in the brain. According to present study the changes in plasma concentrations of androstane and pregnane derivatives are profoundly changed after finasteride treatment. The altered ratio of 5α/5β-steroids is a logical consequence of the action of 5α-reductase inhibitors, as was demonstrated earlier [18, 19] by determination of steroids excreted in urine. Here we demonstrate that it concerns a broad spectrum of testosterone and progesterone metabolites as products of oxido-reduction metabolic pathways. Finasteride treatment exerted also effect on the concentration of hydroxy-metabolites, which seem to be important for the brain function as e.g. 7-hydroxyderivatives of DHEA or pregnenolone [20]. The more detailed knowledge of the potential neuroactive steroids seems to be useful, as the action of individual metabolites differs not only according to their chemical structure but also whether they act on $GABA_A$ or NMAD receptors.

Finasteride might induce depressive symptoms, especially amongst patients who are more susceptible for the disease and to the mechanism of these side-effects the changes in the concentration of known or potential neuroactive steroids should be imputed. Therefore, its medication should be prescribed cautiously for patients with high risk of depression.

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