Laboratory Monitoring of Androgenic Activity in Benign Prostate Hypertrophy Treated with a 5α-Reductase Inhibitor

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Testosterone and androstenedione are metabolized by 5α- and 5β-reductases to androsterone (A) and etiocholanolone (E), respectively. These are excreted in the urine as conjugates, and the A/E ratio in normal men is usually ≥1.5 (as opposed to 1 in women) because of the high 5α-reductase activity in the prostate. The A/E ratio can be determined simply by gas chromatography after acid hydrolysis of a urine sample, extraction of steroids, and formation of trimethylsilyl derivatives. A timed collection of urine is unnecessary because the ratio of A/E is used rather than absolute values. In men suffering from benign prostate hypertrophy who are treated with Finasteride (a 5α-reductase inhibitor), the A/E ratio decreases to <0.5. The A/E ratio decrease can be detected long before there is clinical improvement.

Pharmacological treatment of benign prostate hypertrophy (BPH) has been concerned with reducing androgenic stimulation of the prostate gland.4 Merck, Sharp and Dohme (West Point, PA) introduced a series of 4-aza-steroids (1) that are potent inhibitors of 5α-reductase. The rationale behind this treatment is that 5α-dihydrotestosterone is a much more potent androgen than is testosterone itself and, therefore, prevention of conversion of testosterone to the reduced form should minimize the androgenic stimulus to the prostate.

In the past, assessment of the efficacy of the drug depended entirely on clinical urological investigations. Because the effect on the prostate may not be apparent until several months of treatment have elapsed, it seemed advisable to develop a simple laboratory test to measure the lowering of 5α-reductase activity, if only to ascertain patient compliance in taking the inhibitor. In common with Shackleton et al. (2, 3), we have used the androsterone/etiocholanolone (A/E) ratio in urine as a measure of 5α-reductase activity in hirsutism (4). This measure was used previously by Zumoff et al. (5) to show variations in metabolism of testosterone according to age and sex: the mean A/E ratio for men ages 14–45 years was ~1.5 but fell to 1.0 or even slightly lower in elderly men (ages 69–87 years). The ratio for women was appreciably lower than that for men for each age group.

The purpose here was to determine whether measurement of the A/E ratio could be useful in assessing the effect of a 5α-reductase inhibitor. Because androsterone is formed through 5α-reduction of both testosterone and androstenedione, whereas etiocholanolone is made via 5β-reduction, the 5α-reductase inhibitor ought to considerably lower the A/E ratio.

Materials and Methods

Finasteride (MK-906), the drug used in this study, is (6α,17β)-(1,1-dimethylethyl)-3-oxo-4-azaandrost-1-ene-17-carboxamide. This compound (Proscar, Merck, Sharp & Dohme) is a 4-aza steroid competitive inhibitor of human 5α-reductase. Inhibition of 5α-reductase results in a decrease in target organ concentrations of dihydrotestosterone (DHT). Three conditions are the principal potential indications for using this drug: BPH, carcinoma of the prostate, and female hirsutism. In all three conditions, the androgen-responsive target organ is primarily stimulated by DHT. BPH is presently under investigation with this drug.

Patients

As part of an international multicenter study group, we treated 23 elderly BPH patients with Finasteride for a period of up to one year. The study was double-blind, and patients received placebo or 1 or 5 mg of the drug.

All patients were examined clinically twice before treatment began and every month thereafter. Various urological as well as blood indexes were monitored. For this report, patients had to have been in the study for at least one month, and most had been in the study for more than three months. Fresh urine specimens were collected from each patient in the morning (between 0830 and 0930).

At the end of one year, we uncoded the list of patients and divided the results into three groups: group A, patients taking a placebo (age-matched control subjects; 11 men); group B, patients taking 1 mg of drug (6 men); and group C, patients taking 5 mg of drug (11 men). Because the trial was extended on an open basis, five patients who had been originally on placebo were treated with 5 mg of drug (group D), and three patients previously treated for one year with 1 mg of drug were transferred to 5 mg of drug (group E). The A/E ratio was measured after one month of treatment with 5 mg of drug in groups D and E.

Determination of the A/E Ratio

Androsterone and etiocholanolone were measured in fresh urine samples by our previously published method (6) involving gas chromatography of trimethylsilyl derivatives of the steroids after acid hydrolysis of the
conjugates. Briefly, the method is as follows: 0.4 mL of concentrated hydrochloric acid (12 mol/L) is added to 4.4 mL of urine in a 15-mL conical tube fitted with a ground-glass joint. The tube is heated in a boiling water bath for 15 min and then cooled, 0.2 mL of internal standard solution is added (5α-androstan-3α-ol-11,17-dione; 1 g/L ethanol), and the steroids are extracted with 6 mL of ether. The aqueous layer is removed by aspiration and the ether layer is washed, first with 2 mL of 0.5 mol/L sodium hydroxide and then twice with 2 mL of water. The ether is evaporated under a stream of air (in a water bath held at 45 °C). The extract of steroids is silylated by reaction with 0.2 mL of a mixture of hexamethyldisilazane/trimethylchlorosilane/pyridine (3/1/9 by vol) in a stoppered tube for 25 min in an oven maintained at 120 °C. The solution is evaporated at 45 °C under a stream of air, and the residue is dissolved in 50 μL of n-hexane; 3 μL of this solution is injected onto the gas chromatograph (Model 104, Fye, Cambridge, UK), equipped with a hydrogen flame-ionization detector. The conditions for chromatography are as follows: column: 3% OV-225 on Chromosorb W (HP) 80/100 mesh (Fierce Chemicals Co., Rockford, IL); column temperature: 245 °C (isothermal); and detector temperature: 300 °C.

Results

Figure 1 shows values for the A/E ratio of men treated with Finasteride compared with nontreated control subjects. The A/E ratio among treated men was <0.5 in all cases. Indeed, the highest values, 0.49 in group B and 0.44 in group E, were from the same man, who was transferred to the open trial. In the remainder of cases, the ratios were ≥0.38. By contrast, control values were ≥0.63, and the majority were >1.0.

The mean values of the A/E ratio groups A, B, and C, respectively, were 1.25 (SD 0.59), 0.33 (SD 0.08), and 0.29 (SD 0.08). There was a significant difference between control subjects and members of groups B and C (∆P <0.001 with the unpaired Student's t-test). There was no significant difference between groups B and C.

Figure 2 depicts the five men who were switched from placebo to 5 mg of drug (D). As can be seen, a sharp decline was noted in the A/E ratio after one month of treatment. There was a minimal further decrease in A/E ratios in those in group E.

From the approximately equal mean values of the A/E ratio in groups B and C and from the changes shown in Figure 2, it can be seen that 1 mg of Finasteride per day decreases A/E similarly to the 5-mg/day dosage.

Discussion

The A/E ratio is a good measure of 5α-reductase activity relative to the activity of 5α-reductase. The most striking results are those in group D, where patients whose ratio had been determined previously were checked again after taking the drug. These patients were examined on their first visit to the clinic after the transfer from placebo to drug. The time interval was approximately one month, and it is possible that a decreased A/E ratio could be discerned after less time. However, a check on the efficacy of the drug regarding changes in A/E ratio after only one month is much more striking than the urologic examination, which requires several months for significant clinical changes to appear. Therefore, this test may have several clinical applications, including ensuring patient compliance. The drug will have to be taken for prolonged periods of time, if not indefinitely, to prevent the prostate from growing, and such a simple, noninvasive test to assess compliance will be useful.

The exact dosage of Finasteride to be administered to patients (1 or 5 mg/day) is still under investigation. Determining A/E ratios in patients may help decide the dosage necessary for clinical changes in the prostate long before these changes become clinically overt. There is apparently no appreciable difference between a 1-mg and a 5-mg dosage for decreasing the 5α-reductase activity in normal elderly men. Implementation of the suggested test on a larger group of patients will provide the information regarding the magnitude of 5α-reductase inhibition and the clinical outcome.

Because the analytical result is expressed as a ratio,
absolute values are not required and random sampling of urine is sufficient for assessing the ratio of 5α-/5β-reductase activity. The analysis itself requires, simply, acid hydrolysis of steroid conjugates, ether extraction, trimethylsilylation of the steroids, and injection onto a gas chromatograph (together with internal standard).

During the course of this work, publications appeared in which the effect of Finasteride on urinary steroid metabolites (7) and on blood concentrations (8) was determined. The former work presented results for the A/E ratio similar to those that we obtained. The methodology used included enzyme hydrolysis of conjugates and gas chromatography/mass spectrometry of derivatized steroids. This more sophisticated analysis was perhaps necessary for measuring a whole range of other steroid metabolites. However, for routine analysis of the A/E ratio, our simplified technique has proven to be entirely satisfactory.

In conclusion, measuring the A/E ratio in urine is a simple and efficient way of monitoring 5α-reductase activity. This method might be useful for selecting patients for 5α-reductase inhibitor treatment, to optimize results as well as to monitor patient compliance.

References