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# THE HMG-CoA REDUCTASE INHIBITOR SIMVASTATIN SUPPRESSES HUMAN TESTICULAR TESTOSTERONE SYNTHESIS *IN VITRO* BY A SELECTIVE INHIBITORY EFFECT ON 17-KETOSTEROID-OXIDOREDUCTASE ENZYME ACTIVITY

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**Summary**—In concentrations probably exceeding those achieved *in vivo*, the cholesterol lowering compound simvastatin was found to suppress the synthesis of the androgens androstenediol and testosterone *in vitro* by human testicular homogenates. It was demonstrated that simvastatin in addition to its known inhibitory effect on HMG-CoA reductase activity, also affects the later steps of testicular steroidogenesis by selectively inhibiting the 17-ketosteroid-oxidoreductase catalyzed conversion of dehydroepiandrosterone and androstenedione to androstenediol and testosterone respectively. There was no effect of simvastatin on the Cytochrome *P*-450-dependent microsomal enzymes. Although in doses conventionally used in the treatment of hypercholesterolemia, simvastatin does not affect testicular steroidogenesis, at higher doses—especially when inadvertently administered during early pregnancy—adverse effects on normal testosterone biosynthesis and thereby fetal development should be considered.

Simvastatin [1,2,6,7,8,8α-hexahydro-β-δ-dihydroxy-2,6-dimethyl-8-(2,2-dimethyl-1-oxybutyoxy)-1-naphthalene heptanoic acid-lactone] is a member of a relatively new class of drugs used to treat hypercholesterolemia. These drugs interfere with the biosynthesis of cholesterol through a direct inhibitory effect on 3-hydroxymethyl-glutaryl coenzyme A(HMG-CoA) reductase activity, which catalyzes the rate limiting step in the synthetic process [1, 2]. Related drugs as lovastatin and (more recently) pravastatin have been reported to exert no significant effect on basal or ACTH stimulated cortisol production at doses that lowered LDL–cholesterol [1–5]. However, a trend toward reduced basal plasma testosterone levels has been reported in lovastatin and pravastatin treated patients [1, 5, 6] *In vitro*, Engelhardt *et al.* [7] recently found a dose-dependent inhibition by lovastatin of androstenedione production by porcine ovarian theca cells at the level of the 17α-hydroxylase/C17.20 lyase complex, which could not be restored by addition of 25-hydroxy-cholesterol. This suggests that lovastatin in addition to its HMG-CoA inhibitory effect also affects the later steps in gonadal steroidogenesis. These data prompted us to investigate whether the related cholesterol lowering compound simvastatin also interferes with *in vitro* steroid biosynthesis by human testicular homogenates.

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*Abbreviations:* P5, pregnenolone, 3β-hydroxy-5-pregnen-20-one; 17-OHP5, 17α-hydroxypregnenolone, 3β,17α-dihydroxy-5-pregnen-20-one; P4, progesterone, 4-pregnene-3,20-dione; 17-OHP4, 17α-hydroxyprogesterone, 17α-hydroxy-4-pregnene-3,20-dione; DHEA, dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one; A4, androstenedione, 4-androstene-3,17-dione; A5, androstenediol, 5α androstene-3β,17β-diol; T, testosterone, 17β-hydroxy-4-androsten-3-one; ADL, androstadienol, 5,16-androstadien-3β-ol; 17-KSOR, 17-ketosteroid-oxidoreductase.

## MATERIALS AND METHODS

The techniques used for the study of *in vitro* metabolism of steroids in human testicular homogenates have been described in detail elsewhere [8, 9]. In short, testis tissue was obtained from 4 men, aged 73–78 yr, who underwent orchidectomy for their prostatic carcinoma. None had taken any medication known to

interfere with steroidogenesis. The testes were decapsulated, homogenized on ice in 0.25 M sucrose buffer and centrifuged for 20 min at 10,000 *g*. The supernatant was diluted with 50 mM phosphate buffer, pH 7.4 to about 3 ml/g testis tissue. Incubation of 100 $\mu$l homogenate with about 0.1 $\mu$g [4-$^{14}$C]pregnenolone in a final volume of 1 ml was performed in air at 32°C in the presence of NAD (final concentration, 0.4 mM) and a NADPH-generating system (final concentrations 0.4 mM NADAP, 4 mM glucose-6-phosphate, and 0.12 U/ml glucose-6-phosphate dehydrogenase). The reaction was terminated by adding ice-cold diethyl ether. Eight tritiated marker steroids—the major steroids pregnenolone (P5), 17-OHpregnenolone (17-OHP5), progesterone (P4), 17-OH progesterone (17-OHP4), dehydroepiandrosterone (DHEA), androstenediol (A5), androstenedione (A4) and testosterone (T) were added to monitor procedural losses. The incubation mixtures were extracted twice with diethyl ether and analyzed by HPLC using a diol column (Hibar Lichrosorb Diol, Merck, Rahway, N.J.; 5 $\mu$m) with a *n*-hexane–isopropanol gradient. Yields are expressed as percentage of total radioactivity added as carbon labeled substrate. Simvastatin (gift from Merck, Sharpe & Dohme Inc., Rahway, N.J.)—dissolved in alcohol—was added to the incubation medium in concentrations of 0, 0.01, 0.1, 1, 3, 10, 30 and 100 $\mu$M. In two experiments [$^3$H]DHEA or [$^3$H]A4 (about 20,000 cpm) were used as substrate in stead of [$^{14}$C]P5. Simvastatin was added to the incubation medium in concentrations of 0, 10 and 100 $\mu$M. In most experiments the time intervals 12 and 42 min after sterting incubation were chosen, as maximum 17-OHP5 and DHEA changes occur within 15 min and maximum 17-OHP4, A5 and T changes after about 45 min [8].

Statistical analysis was performed by testing the means of Spearman rank correlation coefficients. The means $\pm$ 1 SD are given.

## RESULTS

In concentrations of 0.01 and 0.1 $\mu$M, simvastatin did not affect steroid synthesis (data not shown). From 1 $\mu$M on, however, a dose-dependent increase of DHEA ($P < 0.02$) and A4 ($P < 0.005$) levels was observed and a reciprocal decrease of A5 ($P < 0.001$) and T ($P < 0.001$), both at $t = 12$ and 42 min after starting the incubation (Figs 1 and 2). At the highest concentration of 100 $\mu$M of simvastatin, the fall of A5 was 97 $\pm$ 4% and 97 $\pm$ 5% at $t = 12$ and 42 min respectively, the fall of T 80 $\pm$ 25 and 71 $\pm$ 29%. The ratios A5/DHEA ($-97 \pm 5\%$ and $-99 \pm 2\%$) and T/A4 $-98 \pm 2\%$ and $-99 \pm 2\%$ respectively at both time intervals) dramatically decreased to virtually zero at the highest concentration (Fig. 1). Reflecting the suppression of 17-ketosteroid-oxidoreductase enzymatic activity the ratio A5 + T over DHEA + A4 sharply fell ($P < 0.005$) to about zero by increasing the concentration of simvastatin.

The remaining major steroids were unaffected by simvastatin. The ratios P5/17-OHP5 (reflecting 17$\alpha$-hydroxylase activity), 17-OHP5/DHEA (17.20 lyase), 17-OHP5/17-OHP4 (3$\beta$-hydroxysteroid dehydrogenase) and P5/androstadienol (16-ene-synthetase activity) were virtually similar at simvastatin concentrations of 0, 10 and 100 $\mu$M (coefficients of variation respectively 7, 6, 4 and 7%).

Using the immediate precursor of A5 [$^3$H]DHEA as a substrate, an increase in the concentration of simvastatin lowered both A5 ($-84\%$ at 10 $\mu$M, $-95\%$ at 100 $\mu$M) and T ($-60$ and $-90\%$ respectively) at $t = 12$ min. The ratios DHEA/A5 and A4/T about 10-fold increased at 10 $\mu$M, and 30 respectively 200 times at 100 $\mu$M. Using the immediate precursor of T [$^3$H]A4 as a substrate the A4/T ratio similarly increased (respectively 10- and 200-fold at 10 and 100 $\mu$M of simvastatin) (Fig. 2).

## DISCUSSION

The data in the present study indicate that from concentrations of 1 $\mu$M on, simvastatin—in addition to the known inhibitory effect on HMG-CoA reductase—interferes with the 17-ketosteroid-oxidoreductase (17-KSOR) catalyzed conversion of DHEA and A4 to respectively A5 and T in human testicular homogenates. The cytochrome *P*-450 linked conversions (17-hydroxylase, 17,20 lyase, and 16-ene-synthetase) remained virtually unaffected. These latter findings are in contrast with recent data of Engelhardt *et al.* [7] who demonstrated an inhibitory effect of the related cholesterol lowering compound lovastatin on the 17-hydroxylase–17,20 lyase complex in cultured ovarian theca cells from prepubertal girls. We have no explanation for this discrepancy in results between theirs and our data except for differences in species, age, the gonadal

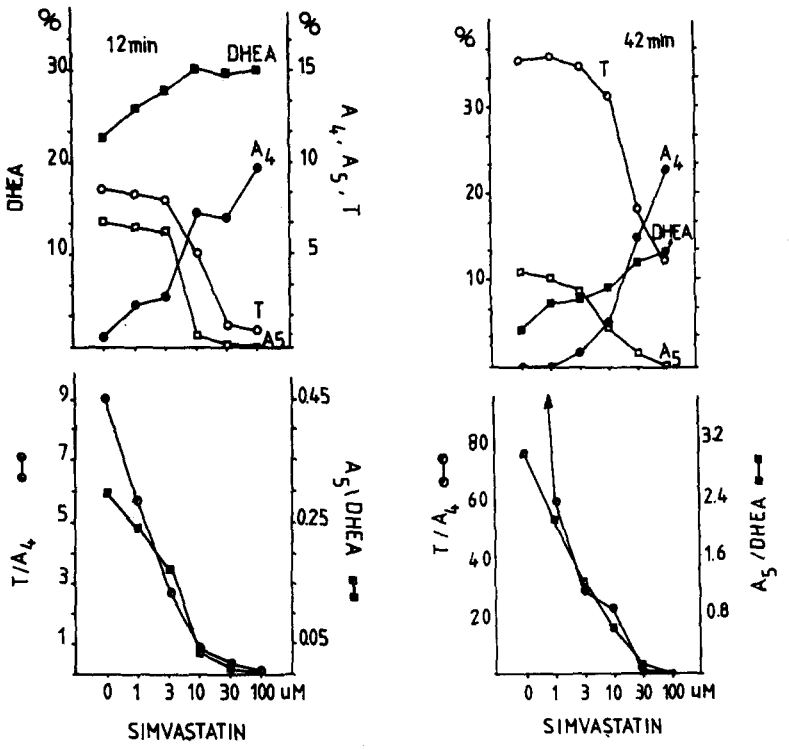


Fig. 1. Effect of increasing concentrations of simvastatin on DHEA, A4, A5 and T synthesis from [4-$^{14}$C]P5 in human testicular homogenates and on the ratio A5/DHEA and T/A4 at 12 (left panel) and 42 min (right panel) after starting the incubation. For the sake of clarity the means without SD have been given. Note that in the right upper panel the scale is the same for all steroids.

material used and differences in chemical structure between both HMG-CoA inhibitors. It appears from our data, that the suppression of A5 and especially T synthesis is not complete even at the highest concentration of 100 $\mu$M of simvastatin. At the clinically useful doses of 20 mg once or twice daily inhibitory concentrations of simvastatin as obtained in our *in vitro* study probably never will be achieved [7].

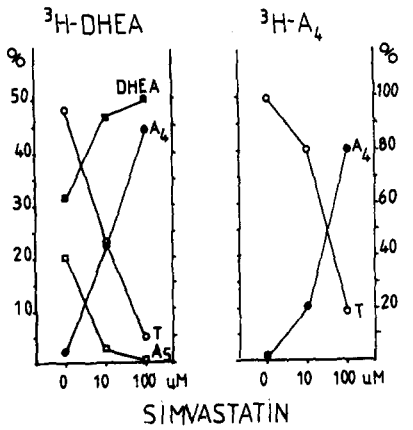


Fig. 2. Effect of 0, 10 and 100 $\mu$M simvastatin on A4 and/or T synthesis from respectively [$^3$H]DHEA (left panel) and [$^3$H]A4 (right panel) in human testicular homogenates at $t$ = 12 min after starting the incubation.

As a consequence of extensive first pass extraction in the liver less than 5% of the lactone—used in our study—or its active open $\beta$-hydroxy acid derivate (L 654.969) will reach the systemic circulation [10]. For lovastatin the mean plasma levels of the total inhibitor reportedly are about 0.2 $\mu$M Equiv. at the maximum recommended dose of 80 mg given once daily [10]. It seems therefore plausible that only at doses four or five times exceeding those currently used in clinic adverse effects of simvastatin on testicular steroidogenesis can be expected.

The mechanism by which simvastatin—at least the lactone compound—exerts its inhibitory effect on microsomal 17-KSOR activity and thereby on A5 and T production in human testicular homogenates is not known. This inhibition is rather selective as the microsomal cytochrome *P*-450 catalyzed conversions remained unaffected. Simvastatin has no apparent chemical and structural relationship to drugs known to interfere with oxidoreductase such as non-steroidal anti-inflammatory drugs (zomepirac, flufenamic acid and indomethacine) or non-steroidal estrogens. Probably their important part for binding to the enzyme is the structure resembling the steroid ring [11].

It cannot be excluded that a similar mechanism may be operative in the case of simvastatin, which possesses 2 steroid rings.

Summarizing, the data demonstrate that simvastatin in supratherapeutical concentrations inhibits human testicular testosterone synthesis *in vitro* through a direct effect on the 17-ketosteroid-oxidoreductase enzyme. At doses conventionally used in the treatment of hypercholesterolemia simvastatin does not affect testicular steroidogenesis *in vivo*. However, at higher doses—especially when inadvertently administered during early pregnancy—adverse effects on testosterone synthesis and thereby on fetal development should be considered.

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