Influence of Simvastatin, Pravastatin, and BM 15.766 on Neutral Sterols in Liver and Testis of Guinea Pigs

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There is mounting evidence that specific sterol precursors of cholesterol are associated with reproductive functions. Testicular meiosis-activating sterol (T-MAS) and follicular fluid meiosis–activating-sterol (FF-MAS) are 2 cholesterol precursors found in reproductive organs of mammals, which are able to overcome meiotic arrest in vitro. This study investigates the influence of simvastatin and pravastatin, 2 inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, and BM 15.766, an inhibitor of 7-dehydrocholesterol reductase, on concentrations of neutral sterols in liver and testis of guinea pigs. Concentrations of T-MAS, lathosterol, and desmosterol were markedly higher in testis compared with liver. Simvastatin (150 mg/d) and pravastatin (150 mg/d and 350 mg/d) markedly reduced cholesterol precursors in liver. In contrast, T-MAS and desmosterol in testis remained unchanged, albeit other cholesterol precursors were reduced. BM 15.766 led to the accumulation of 7- and 8-dehydrocholesterol, both in liver and testis. However, concentrations of T-MAS in testis were not changed by BM 15.766. We conclude that treatment of guinea pigs with simvastatin, pravastatin, or BM 15.766, which simulates the biochemical defect of the Smith-Lemli-Opitz (SLO) syndrome, does not affect T-MAS concentrations in testis.

THE CONVERSION OF 3β-hydroxy-3β-methylglutaryl coenzyme A to mevalonic acid by the enzyme 3β-hydroxy-3β-methylglutaryl-CoA reductase (HMG-CoA reductase) is considered to be the rate-limiting step in cholesterol synthesis (Fig 1). Within this cascade, lanosterol is the first sterol intermediate (Fig 1), and further conversion of lanosterol to cholesterol involves 19 steps, resulting in numerous sterol intermediates.[1] Only very low concentrations of these sterol precursors of cholesterol are found in blood and tissues of mammals. For a long time, no biologic functions had been associated with these sterols. However, in 1995, Byskov et al[2] discovered that 2 sterols isolated from human follicular fluid (4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol [FF-MAS]) and from bull testis (4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol [T-MAS]) possess meiosis-activating capacity. FF-MAS and T-MAS are present in relatively high concentrations in gonads of mammals[3] as precursors of cholesterol (Fig 1).[1] This discovery was the first report of a major biologic activity of sterol precursors of cholesterol. In addition, Connor et al[4] found a marked increase of the cholesterol precursor, desmosterol, in monkey testis during puberty and reported high concentrations of desmosterol in monkey and human spermatozoa.[5-7] Desmosterol has also been described to be present in high concentrations in the epididymis of several mammalian species.[8-11] The cholesterol precursor, cholesta-7,24-dien-3β-ol, has been found to be a major sterol in hamster cauda epididymis.[12,13]

HMG-CoA reductase inhibitors are cholesterol-lowering drugs used for the treatment of hypercholesterolemia.[14-17] Most HMG-CoA reductase inhibitors currently available are lipophilic drugs (eg, simvastatin), which are able to cross membrane layers by passive diffusion and therefore influence cholesterol synthesis not only in liver, but also in extrahepatic tissues.[18-20] In contrast, effects of pravastatin, a more hydrophilic HMG-CoA reductase inhibitor, are regarded as more liver specific, due to a selective uptake in liver, which is facilitated by a multispecific anion transporter[21] and resuls in a smaller effect on cholesterol synthesis in extrahepatic tissues compared with simvastatin.[18]

BM 15.766 inhibits the activity of 7-dehydrocholesterol reductase, an enzyme catalyzing the ultimate step in cholesterol biosynthesis: the conversion of 7-dehydrocholesterol to cholesterol (Fig 1).[22,23] A markedly decreased activity of 7-dehydrocholesterol reductase has been identified as the biochemical disorder of the autosomal inherited Smith-Lemli-Opitz (SLO) syndrome, leading to high serum concentrations of dehydrocholesterols.[24,25] Therefore, BM 15.766 is frequently used to simulate this disease in animal models and cell culture experiments to investigate the effect(s) of blocking cholesterol biosynthesis at this distinct step and to evaluate possible treatment regimes.[26-30]

The present study investigates the hypotheses that simvastatin, pravastatin, and BM 15.766 influence neutral sterols in liver and testis, including the putative signaling molecules, T-MAS and FF-MAS.

MATERIALS AND METHODS

Materials

If not otherwise stated, chemicals were purchased from Sigma (Deisenhofen, Germany) in the highest available purity. T-MAS and FF-MAS were generously donated by Drs G.J. Schroepfer Jr and W.K. Wilson (Houston, TX).

Animals

Adult male guinea pigs (Dunkin Harfley; age, 6 to 8 weeks; body weight, $\approx$ 400 g) were obtained from Harlan Winkelmann (Borchen, Germany) and were maintained in darkness from 6 PM to 6 AM and light

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Submitted June 23, 2001; accepted October 10, 2001.

Supported by Grant No. BMFT 01EC9402 from the Bundesministerium für Forschung und Technologie.

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0026-0495/02/5104-0022$35.00/0

doi:10.1053/meta.2002.31341

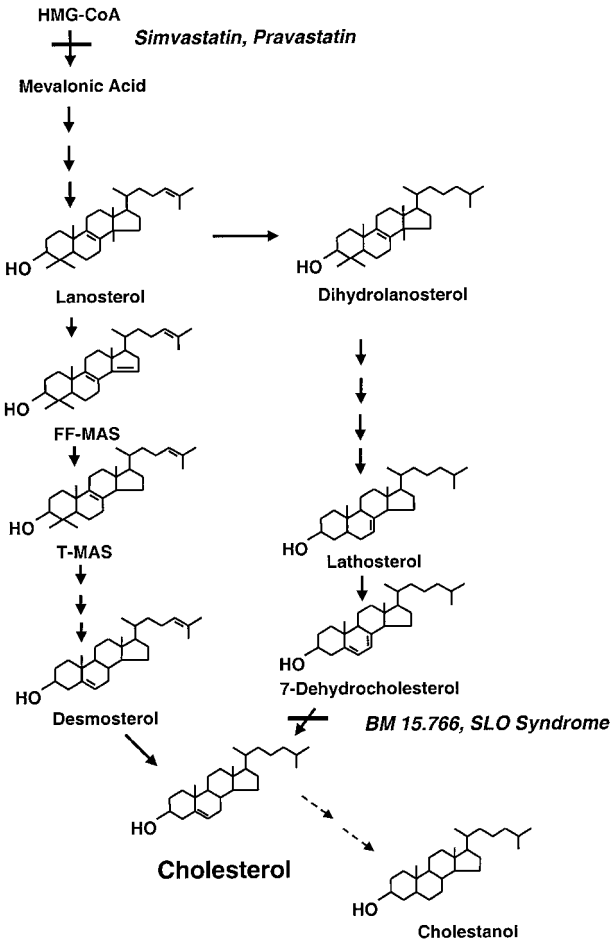

Fig 1. Late steps of cholesterol synthesis.

from 6 AM to 6 PM alternatingly. Free access to water and food was provided. The animals were fed with a standard laboratory chow (Harlan Teklad FD2 Guinea-Pig Diet 9610; Harlan-Winkelmann GmbH, Borchen, Germany). The diet was supplemented with simvastatin (0.5%; Merck Sharp and Dohme, Rahway, NJ) or BM 15.766 (0.066%; Roche Diagnostics GmbH, Mannheim; Germany). Based on approximately 30 g dietary intake of the chow, animals received 150 mg/d simvastatin and 20 mg/d BM 15.766. Pravastatin (Bristol-Myers-Squibb, Regensburg, Germany) was dissolved in Ringer's solution (low dose, 150 mg/d in 200 μL and high dose, 350 mg/d in 400 μL) and given orally. Treatment periods lasted 3 weeks. The animals were weighed, and food intake was controlled for all groups on a daily basis. At the end of each experiment, the animals were killed after anesthesia with 150 mg/kg/body weight Ketamin (Hostaket Hoechst, Bad Soden, Germany) and 5 mg/kg/body weight Xylazin (Rompun 2%; Bayer, Leverkusen, Germany). Blood was collected by intracardial punctation. Liver and testis were immediately frozen in liquid nitrogen and stored at –20°C until sample preparation. The animal study was approved by the animal welfare legislation.

Sterol Extraction and Analysis

Testis and liver were homogenized and diluted 1:1 (wet wt/vol) with distilled water. Neutral sterols were extracted in hexane/isopropanol (3:2 vol/vol; twice with 4 mL) from homogenates corresponding to 50 mg testis wet weight. Epicoprostanol (1 μg; stock solution 20 ng/μL in hexane) and 5α-cholestan (50 μg; stock solution 1 μg/μL in hexane) were added as internal standards. The solvents were removed by a gentle stream of nitrogen, and an alkaline hydrolysis was performed with 1 mL of 1 N NaOH in 80% ethanol for 1 hour at 70°C. After cooling to room temperature and addition of 0.5 mL bidistilled water, neutral sterols were extracted twice into 3 mL cyclohexane (vortexing for 30 seconds, centrifugation for 10 minutes at 2,500 rpm). The organic phases were combined and evaporated by a stream of nitrogen. The trimethylsilyl (TMS) derivatives of sterols were formed by adding 100 μL bis-trimethylsilyl-trifluoracetamide (BSTFA)/n-decane (1:1; vol/vol) for 1 hour at 70°C. A total of 1 to 2 μL was used for gas-chromatography/mass-spectrometry (GC/MS) or gas-chromatography/flame-ionization detection (GC/FID).

Cholesterol was additionally measured in serum by routine clinical chemistry methods using the flex reagent cassette (Dimension Clinical Chemistry System; DadeBehring, Liederbach, Germany).

GC/MS

GC/MS was performed on a Hewlett-Packard GC-MS system (5890 series II GC combined with a 5971 mass selective detector) equipped with a DB-XLB (30 m $\times$ 0.25 mm id $\times$ 0.25 μm film; J&W, Palo Alto, CA) in the splitless mode using helium (1 mL/min) as carrier gas. The temperature program was as follows: 150°C for 1 minute, followed by 20°C/minute up to 260°C, and 10°C/minute up to 280°C (for 15 minutes). The electron-multiplier voltage was raised by 300 V above the autotune value after the elution of lathosterol to increase sensitivity for sterols eluting thereafter. Data were collected by selective ion monitoring (SIM) and the full-scan mode (range, *m/z* 50 to 550) was used for compound identification using authentic reference compounds.

Neutral sterols were monitored as their TMS derivatives in the SIM mode using the following masses: desmosterol (*m/z* 456, 441, and 351); lathosterol (*m/z* 458); lanosterol (*m/z* 498 and 393); T-MAS (*m/z* 484); FF-MAS (*m/z* 482); cholestanol (*m/z* 460 and 306); campesterol (*m/z* 472); and sitosterol (*m/z* 486). The internal standards epicoprostanol and 5α-cholestane were measured on *m/z* 370 and 372, respectively. Peak integration was performed manually, and sterols were quantified from SIM analyses against the internal standards using standard curves for the listed sterols.

GC/FID

Cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol were quantified by GC/FID on a Hewlett-Packard (Palo Alto, CA) GC-system (HP 6890 series II plus GC). The GC-system operated in the splitless mode (injection temperature, 280°C) using hydrogen (1.1 mL/min) as carrier gas. Sterols were separated on a DB-XLB (30 m $\times$ 0.25 mm id $\times$ 0.25 μm film, J&W) using the following temperature gradient: 150°C for 3 minutes, heating by 30°C/minute up to 290°C (for 22 minutes). Cholesterol and the 2 dehydrocholesterols were quantified as TMS-derivatives. Thus, the ratios of the sterol areas to the area of the internal standard were calculated and multiplied by the added amount of the internal standard.

Statistics

Results are given as mean $\pm$ SD. Differences were considered significant at a level of $P < .05$, calculated by Student's t test for independent samples.

RESULTS

Neutral Sterols in Liver and Testis

Figure 2 shows GC/MS chromatograms of neutral sterols monitored by their specific ions in liver and testis of guinea pigs, revealing that the cholesterol precursor profiles were

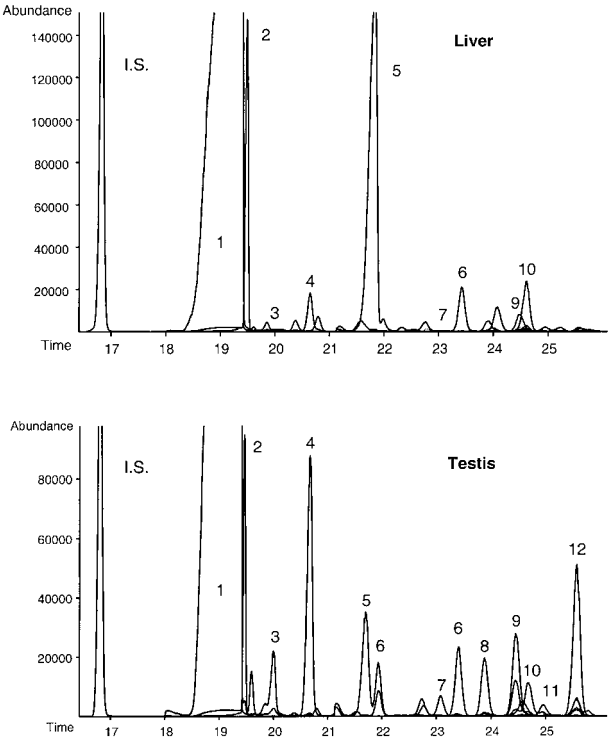

Fig 2. Neutral sterols in liver and testis of guinea pigs analyzed by GC/MS in the selected ion monitoring mode. Neutral sterols were extracted and measured as described in Materials and Methods. Peak identification: IS, internal standard (epicoprostanol, *m/z* 370); 1, cholesterol (*m/z* 458); 2, cholestanol (*m/z* 306); 3, desmosterol (*m/z* 456); 4, lathosterol (*m/z* 458); 5, campesterol (*m/z* 472); 6, monomethylsterols (*m/z* 472), 7, dihydrolanosterol (*m/z* 395); 8, dimethylsterol (*m/z* 486); 9, lanosterol (*m/z* 393); 10, sitosterol (*m/z* 486); 11, FF-MAS (*m/z* 482); and 12, T-MAS (*m/z* 484).

markedly different in liver compared with testis. Most obvious are the 2 to 5 times higher concentrations of lathosterol, desmosterol, lanosterol, and dihydrolanosterol in testis compared with liver (Tables 1 and 2). In addition, the cholesterol precursor, T-MAS, was barely detectable in liver, but became a major cholesterol precursor in testis, similar to the concentrations of lathosterol and desmosterol (Fig 2; Tables 1 and 2). FF-MAS was also detectable in testis, but yielded concentrations of only 6% of T-MAS (Fig 2; Tables 1 and 2). Some other monomethyl- and dimethylsterols (molecular ions as TMS-derivatives: *m/z* 472 and 486), which were identified as cholesterol precursors, because of their incorporation of ^{13}C-acetate in cell culture experiments (unpublished data), were also detected in higher concentrations in testis compared with liver (Fig 2). In contrast, the plant sterols, campesterol and sitosterol, were present in testis in concentrations approximately 4 times lower compared with liver. Cholesterol and the cholesterol derivative, cholestanol, showed only minor differences between liver and testis.

Neutral Sterols in Liver After Drug Treatments

Concentrations of cholesterol, cholesterol precursors, and plant sterols in liver of guinea pigs after different drug treatments are given in Table 1. Cholesterol, as well as concentrations of the cholesterol derivative, cholestanol, did not change during treatment with HMG-CoA reductase inhibitors, but were significantly reduced (-57% for cholesterol and –62% for cholestanol) after treatment with the 7-dehydrocholesterol reductase inhibitor, BM 15.766 (Table 1). Treatments with simvastatin or pravastatin with 2 different dosages were about equally potent in significantly reducing concentrations of the main cholesterol precursors, lathosterol and lanosterol (range, –57% to –82%). Desmosterol and dihydrolanosterol, which are present in liver in lower concentrations than lathosterol and lanosterol, were also reduced. However, these reductions failed to be significant for desmosterol following all 3 treatments with

Table 1. Neutral Sterols in Liver of Guinea Pigs After Different Drug Treatments

	ng/mg (wet weight) (mean ± SD)				
Sterol	Control n = 4	Simvastatin (150 mg/d) n = 4	Pravastatin (350 mg/d) n = 4	Pravastatin (150 mg/d) n = 5	BM 15.766 (20 mg/d) n = 6
Cholesterol (μg/mg)	1.68 ± 0.31	2.04 ± 0.39	1.64 ± 0.14	1.63 ± 0.23	0.72* ± 0.11
Cholestanol	30.8 ± 7.2	32.1 ± 5.4	33.1 ± 5.8	32.5 ± 5.3	11.8* ± 2.8
Lathosterol	5.44 ± 1.57	1.66† ± 0.29	1.55† ± 0.10	2.01† ± 0.98	13.64* ± 3.14
Desmosterol	0.55 ± 0.21	0.35 ± 0.10	0.36 ± 0.07	0.48 ± 0.27	ND
7-Dehydrocholesterol	Trace	Trace	Trace	Trace	654 ± 62.2
8-Dehydrocholesterol	Trace	Trace	Trace	Trace	91.2 ± 24.2
Dihydrolanosterol	0.11 ± 0.03	0.03† ± 0.01	0.03† ± 0.01	0.08 ± 0.06	0.03* ± 0.01
Lanosterol	0.84 ± 0.28	0.20† ± 0.04	0.15† ± 0.04	0.36‡ ± 0.21	0.33† ± 0.17
T-MAS	Trace	Trace	Trace	Trace	Trace
FF-MAS	Trace	Trace	Trace	Trace	Trace
Campesterol	55.6 ± 6.1	53.1 ± 4.0	53.6 ± 14.5	48.6 ± 10.8	48.9 ± 6.8
Sitosterol	8.5 ± 4.4	8.4 ± 2.6	7.1 ± 2.6	5.8 ± 1.1	9.6 ± 1.3

NOTE. μg/mg for cholesterol.

Abbreviation: ND, not detectable.

*$P < .001$.

†$P < .01$.

‡$P < .05$.

Table 2. Neutral Sterols in Testis of Guinea Pigs After Different Drug Treatments

Sterol	ng/mg (wet weight) (mean ± SD)				
	Control n = 4	Simvastatin (150 mg/d) n = 4	Pravastatin (350 mg/d) n = 4	Pravastatin (150 mg/d) n = 5	BM 15.766 (20 mg/d) n = 6
Cholesterol (μg/mg)	2.14 ± 0.34	2.06 ± 0.10	1.94 ± 0.08	1.94 ± 0.14	0.86* ± 0.18
Cholestanol	26.1 ± 3.8	24.6 ± 4.3	22.8 ± 3.8	23.1 ± 4.5	14.6† ± 4.1
Lathosterol	23.5 ± 2.7	18.5‡ ± 1.6	14.8† ± 2.3	19.4§ ± 2.2	31.3† ± 2.9
Desmosterol	21.4 ± 7.9	11.5 ± 5.6	22.1 ± 9.4	27.9 ± 14	ND
7-Dehydrocholesterol	Trace	Trace	Trace	Trace	950 ± 99
8-Dehydrocholesterol	Trace	Trace	Trace	Trace	48 ± 12
Dihydrolanosterol	0.23 ± 0.03	0.14‡ ± 0.05	0.12† ± 0.03	0.22 ± 0.06	0.29‡ ± 0.04
Lanosterol	2.76 ± 0.48	1.70‡ ± 0.40	1.80‡ ± 0.17	2.50 ± 0.52	2.52 ± 0.42
T-MAS	15.6 ± 2.6	10.0 ± 5.0	15.2 ± 2.7	16.6 ± 7.1	12.2 ± 2.8
FF-MAS	1.01 ± 0.27	0.66 ± 0.11	0.81 ± 0.07	0.83 ± 0.16	1.58‡ ± 0.30
Campesterol	13.9 ± 1.2	8.4† ± 1.5	9.7‡ ± 1.5	9.8 ± 3.5	9.2† ± 1.5
Sitosterol	2.4 ± 0.42	2.2 ± 0.12	2.0 ± 0.15	2.0 ± 0.84	1.9 ± 0.22

NOTE. μg/mg for cholesterol.
Abbreviation: ND, not detectable.
*$P < .001$.
†$P < .01$.
‡$P < .05$.
§$P = .053$.

HMG-CoA reductase inhibitors and for dihydrolanosterol after treatment with low dose pravastatin (Table 1). Treatment with BM 15.766 led to the accumulation of 7- and 8-dehydrocholesterol and increased lathosterol 2.5-fold. In contrast, lanosterol and dihydrolanosterol were significantly reduced by BM 15.766 (Table 1). Concentrations of the plant sterols, campesterol and sitosterol, in liver were not changed during either HMG-CoA reductase inhibitors or BM 15.766 treatments (Table 1).

Neutral Sterols in Serum After Drug Treatments

All treatment regimes reduced serum cholesterol concentrations significantly (control, 53 ± 9 mg/dL; simvastatin, 150 mg/d, 13 ± 2 mg/dL; pravastatin, 150 mg/d, 29 ± 5 mg/dL; pravastatin, 350 mg/d, 13 ± 1 mg/dL, $P < .01$ for all treatments *v* control). BM 15.766 reduced the concentrations of neutral sterols in serum to 15 ± 3 mg/dL, $P < .001$ versus control. However, after BM 15.766 treatment, 7- and 8-dehydrocholesterol accounted for about 30% of neutral sterols, as assessed by GC/FID analysis.

Neutral Sterols in Testis After Drug Treatments

Cholesterol and cholestanol also remained unchanged in testis after treatments with HMG-CoA reductase inhibitors, but were significantly lowered by more than 50% after treatment with BM 15.766 (Table 2). Lathosterol, lanosterol, and dihydrolanosterol were significantly reduced in testis by simvastatin and high-dose pravastatin treatment (range, -35% to -48%), but remained unchanged by low-dose pravastatin (Table 2). In contrast, concentrations of desmosterol, T-MAS, and FF-MAS were not significantly changed by the different HMG-CoA reductase inhibitors (Table 2). As in the liver, BM 15.766 treatment led to the accumulation of 7- and 8-dehydrocholesterol and significantly increased concentrations of lathosterol, FF-MAS, and dihydrolanosterol, whereas lanosterol and T-MAS were not changed (Table 2). Significant reductions were observed for campesterol after treatment with simvastatin, high-dose pravastatin, and BM 15.766 (range, -29% to −40%). However, slight reductions of sitosterol failed to be significant during all drug treatments (Table 2).

Proportions of Neutral Sterols in Liver and Testis After Drug Treatments

Calculating the ratios of neutral sterols to cholesterol in liver and testis shows proportional changes of noncholesterol sterols after drug treatments. Results are given in Table 3 for liver and Table 4 for testis. Observed changes for treatments with HMG-CoA reductase inhibitors correspond with changes described in Tables 1 and 2. In contrast, the ratios of neutral sterols to cholesterol are markedly increased (except cholestanol) after BM 15.766 treatment, which is due to the more than 50% reduction of cholesterol in both tissues (Tables 3 and 4). Therefore, proportions of the individual sterols in relationship to the total sterol concentration has markedly changed after BM 15.766 treatment in liver and testis.

DISCUSSION

Recent investigations point to putative functions of cholesterol precursors in mammalian fertility, albeit their biologic importance remains to be shown conclusively. Byskov et al[2] discovered that 2 cholesterol precursors isolated from bull testis and human follicular fluid (T-MAS and FF-MAS) were able to overcome meiotic arrest in vitro and considered to be the long sought meiosis-inducing substances.[3,31,32]. After this pioneering work, most studies investigated the role of FF-MAS in fertility, eg, revealing the upregulation of FF-MAS concentrations by follicle-stimulating hormone (FSH) and the beneficial effects of FF-MAS on oocyte maturation.[3,33,34] Even fewer data are available on T-MAS. T-MAS appears in high concentrations in gonads of several species and increases by about

Table 3. Ratios of Neutral Sterols to Cholesterol in Liver of Guinea Pigs After Different Drug Treatments

Sterol	ng/μg (mean + SD)				
	Control n = 4	Simvastatin (150 mg/d) n = 4	Pravastatin (350 mg/d) n = 4	Pravastatin (150 mg/d) n = 5	BM 15.766 (20 mg/d) n = 6
R-Cholestanol	18.6 ± 4.9	15.9 ± 2.2	20.2 ± 3.4	20.0 ± 0.9	16.3 ± 2.1
R-Lathosterol	3.29 ± 1.01	0.84* ± 0.24	0.95* ± 0.11	1.28† ± 0.71	18.96‡ ± 2.90
R-Desmosterol	0.33 ± 0.10	0.17† ± 0.02	0.22 ± 0.04	0.30 ± 0.19	ND
R-7-Dehydrocholesterol	Trace	Trace	Trace	Trace	926 ± 157
R-8-Dehydrocholesterol	Trace	Trace	Trace	Trace	129 ± 43
R-Dihydrolanosterol	0.066 ± 0.02	0.016* ± 0.01	0.018* ± 0.01	0.049 ± 0.04	0.046 ± 0.02
R-Lanosterol	0.52 ± 0.16	0.10* ± 0.02	0.09* ± 0.02	0.23† ± 0.15	0.47 ± 0.30
R-T-MAS	Trace	Trace	Trace	Trace	Trace
R-FF-MAS	Trace	Trace	Trace	Trace	Trace
R-Campesterol	33.5 ± 2.9	26.6 ± 5.1	32.5 ± 8.2	29.9 ± 6.2	68.5‡ ± 9.6
R-Sitosterol	4.91 ± 1.9	4.37 ± 2.2	4.43 ± 1.8	3.58 ± 0.77	13.7* ± 3.5

Abbreviation: ND, not detectable.
*$P < .01$.
†$P < .05$.
‡$P < .001$.

4 times during puberty in rat testis.[3,35] Putative functions of T-MAS include enhancing/maintaining meiosis in testis and/or triggering the resumption of the second meiotic division in oocytes after spermatozoa/oocyte fusion.[3] Concentrations of T-MAS given here for guinea pigs are comparable to those reported by Byskov et al[3] for other mammals. Our results show that the concentration of the second meiosis-acitvating sterol discovered from human follicular fluid, FF-MAS, is only about 6% that of T-MAS in the male guinea pigs, which is in accordance with previous reports of higher concentrations for T-MAS versus FF-MAS in male animals of other species.[2,3]

The cholesterol precursor, desmosterol, has been described in high concentrations in monkey testis, increasing by about 40 times during puberty.[4] Desmosterol is also a major sterol in epididymis and spermatozoa of several mammalian species.[4,5,8,10,11,36] To our knowledge, no other cholesterol precursors, such as lathosterol and lanosterol, have been quantified until now in mammalian testis. The results show pronounced differences between cholesterol precursors in liver and testis with notably higher concentrations of T-MAS, desmosterol, and lathosterol in testis. These differences are in accordance with putative functions of cholesterol precursors in fertility, which might involve differential regulation of cholesterol synthesis in testis compared with other organs.

The present study investigated the effects of 2 HMG-CoA reductase inhibitors (simvastatin and pravastatin) and an inhibitor of Δ7-dehydrocholesterol reductase (BM 15.766) on neutral sterols in liver and testis of guinea pigs.

Our results show that simvastatin (150 mg/d), high-dose pravastatin (350 mg/d), and low-dose pravastatin (150 mg/d) were about equally potent in reducing cholesterol precursors in liver, resulting in about equal potency in reducing cholesterol synthesis. These results are in accordance with previous stud-

Table 4. Ratios of Neutral Sterols to Cholesterol in Testis of Guinea Pigs After Different Drug Treatments

Sterol	ng/μg (mean ± SD)				
	Control n = 4	Simvastatin (150 mg/d) n = 4	Pravastatin (350 mg/d) n = 4	Pravastatin (150 mg/d) n = 5	BM 15.766 (20 mg/d) n = 6
R-Cholestanol	12.3 ± 1.0	12.0 ± 2.3	11.7 ± 1.6	11.9 ± 1.9	17.0* ± 2.9
R-Lathosterol	11.1 ± 1.0	9.0* ± 1.1	7.6† ± 0.9	10.0 ± 0.8	37.3‡ ± 5.7
R-Desmosterol	9.7 ± 2.2	5.6 ± 2.7	11.2 ± 4.6	14.2 ± 6.7	ND
R-7-Dehydrocholesterol	Trace	Trace	Trace	Trace	1,135 ± 200
R-8-Dehydrocholesterol	Trace	Trace	Trace	Trace	58 ± 18
R-Dihydrolanosterol	0.11 ± 0.01	0.07* ± 0.03	0.06† ± 0.02	0.11 ± 0.026	0.35‡ ± 0.06
R-Lanosterol	1.30 ± 0.15	0.83* ± 0.21	0.93* ± 0.12	1.28 ± 0.23	2.97‡ ± 0.30
R-T-MAS	7.31 ± 0.47	4.87 ± 2.51	7.81 ± 1.33	8.52 ± 3.52	14.31† ± 2.82
R-FF-MAS	0.47 ± 0.11	0.32 ± 0.06	0.42 ± 0.04	0.43 ± 0.07	1.90† ± 0.57
R-Campesterol	6.58 ± 0.88	4.08† ± 0.85	4.98* ± 0.64	5.05 ± 1.76	10.88† ± 1.81
R-Sitosterol	1.08 ± 0.16	1.06 ± 0.10	1.01 ± 0.05	1.04 ± 0.42	2.31‡ ± 0.39

Abbreviation: ND, not detected.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

ies, which show comparable efficiency of simvastatin and pravastatin on inhibition of cholesterol synthesis in liver and hepatocytes.[18,37,38] In contrast to treatments with HMG-CoA reductase inhibitors, BM 15.766 led to a dramatic change in the sterol composition of the liver. The accumulation of 7-and 8-dehydrocholesterol is consistent with inhibition of Δ7-dehydrocholesterol reductase by BM 15.766 and the subsequent increase of dehydrocholesterols.[26-30] The same accumulation of dehydrocholesterols in serum and tissues is known for SLO patients exhibiting defective Δ7-dehydrocholesterol reductase activity.[24,25] Eight-dehydrocholesterol has not been identified as a cholesterol precursor, but the reversibility of the enzyme Δ8-Δ7-isomerase has been demonstrated,[39,40] giving a rationale for the concomitant appearance of 8-dehydrocholesterol together with high concentrations of 7-dehydrocholesterol, observed in SLO patients, as well as in studies blocking sterol Δ7-reductase by drug treatments.[28,30,41] The increase of lathosterol after BM 15.766 treatment is consistent with the inhibition of cholesterol synthesis, because lathosterol is the precursor of 7-dehydrocholesterol (Fig 1).

Treatment with simvastatin (150 mg/d) and high-dose pravastatin (350 mg/d) showed significant reductions in testis only for lathosterol, lanosterol, and dihydrolanosterol, indicating also reduced cholesterol synthesis in this organ, whereas low-dose pravastatin (150 mg/d) had no effect on neutral sterols. However, T-MAS and FF-MAS were not affected significantly by any treatment with HMG-CoA reductase inhibitors. These results are in line with previous reports that pravastatin is less effective in the inhibition of cholesterol synthesis in extrahepatic tissues and cell cultures compared with simvastatin, whereas both drugs are about equally potent in blocking cholesterol synthesis in hepatocytes.[18,19,37,42-45] The testis-blood barrier might also be involved in diminishing the effect of HMG-CoA reductase inhibitors on cholesterol synthesis in testis.[46,47] The question of a potential influence of treatment with HMG-CoA reductase inhibitors on fertility has been raised previously, but all studies, so far, failed to show significant effects on male reproductive physiology in humans.[48-55] Cell culture experiments also showed no or only minor effects of HMG-CoA reductase inhibitors on reproductive parameters.[56,57] Although we observed reductions in some cholesterol precursors in testis of guinea pigs treated with HMG-CoA reductase inhibitors, the putative signaling MAS compounds remained virtually unaffected in this study. This observation supports the hypothesis that HMG-CoA reductase inhibitors do not alter reproductive physiology mediated by neutral sterols in testis. However, differences in the regulation of cholesterol synthesis in testis compared with liver has been reported.[58,59] This might explain the discrepancy between the reduction of some cholesterol precursors in testis after simvastatin and high-dose pravastatin treatment in testis, but not for T-MAS and FF-MAS. It should be noted that the dose of the HMG-CoA reductase inhibitors used here for guinea pigs (150 and 350 mg/d) markedly exceeds the common dosage for humans (10 to 80 mg/d) if calculated for body weight.

Changes of neutral sterols in testis after BM 15.766 treatment parallels changes in liver. Besides the expected accumulation of dehydrocholesterols, other cholesterol precursors remained unchanged (T-MAS, lanosterol) or even increased (lathosterol, FF-MAS, dihydrolanosterol). However, little is known about the influence of reduced cholesterol and cholestanol and accumulated dehydrocholesterols in the testes on fertility. Development of ambiguous genitalia has been reported in some SLO patients, but hypothalamic-pituitary-gonadal function was not impaired in the few patients investigated so far.[25,60-62] However, these reports partially refer to observations before the biochemical defect of the SLO syndrome was discovered and have, therefore, to be interpreted with caution. Based on the results of the present study, it seems unlikely that there is a major change of MAS compounds in testis of SLO patients, and that possible effects on reproductive physiology are due rather to reduced concentrations of cholesterol and the accumulation of dehydrocholesterols.

Campesterol and sitosterol are 2 major plant sterols. The appearance of plant sterols in mammalian tissues is solely due to their absorption from the diet and their transport in serum lipoproteins. In humans, serum concentrations of plant sterols have been shown to reflect cholesterol absorption efficiency.[63,64] Here, the plant sterols, campesterol and sitosterol, as well as their ratio in liver, showed no differences after treatment with HMG-CoA reductase inhibitors, indicating no influence on cholesterol absorption. In testis, simvastatin and high-dose pravastatin treatment reduced the major plant sterol, campesterol, as well as the ratio of campesterol to cholesterol significantly, whereas reductions for sitosterol, appearing in much lower concentrations, were not significant. This observation is likely to be due to markedly reduced lipoprotein concentrations in plasma, the carriers of cholesterol and other sterols. Therefore, plant sterols uptake by the testis through lipoproteins is likely to be reduced after treatment with HMG-CoA reductase inhibitors. However, increased low-density lipoprotein (LDL) receptor-mediated uptake of lipoproteins might compensate for decreased lipoprotein concentrations in plasma. Furthermore, it cannot be ruled out that the blood-testis barrier discriminates the uptake of different sterols into the testis.

In summary, concentrations of cholesterol precursors in guinea pigs differ markedly between liver and testis with especially high concentrations of T-MAS and desmosterol in testis. Despite their marked influence of cholesterol precursors in liver and the less pronounced reductions of some cholesterol precursors in testis, HMG-CoA reductase inhibitors do not affect MAS compounds in testis. In addition, BM 15.766 leads to an accumulation of dehydrocholesterols in liver and testis similar to the SLO syndrome, but did not change T-MAS and even slightly increased FF-MAS in guinea pig testis. These results do not support effects of these drugs on fertility mediated by neutral sterols in testis.

ACKNOWLEDGMENT

We thank Silvia Winnen and Heike Prange for excellent technical assistance.

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