Cholesterol metabolism during ketoconazole treatment in man

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Abstract Ketoconazole, an antifungal antibiotic, inhibits cholesterol synthesis by blocking demethylation of lanosterol. Effects of this inhibition were studied on serum cholesterol, lipoproteins and cholesterol precursors, biliary lipid composition, and fecal steroid elimination in five patients with prostate cancer treated with large doses of ketoconazole. The serum level of total cholesterol fell by 27%, that of LDL cholesterol by 41% and that of LDL apoB by 32% with ketoconazole alone; the fall in the total cholesterol level of a patient treated with ketoconazole–cholestyramine was 65%. Serum contents of free lanosterol and dihydrolanosterol increased up to 250 times, yet the total concentrations remained <2 mg/dl. Of the other cholesterol precursor sterols only those with Δ^8-double bond increased several times, indicating that in addition to 14α-demethylation, ketoconazole also interfered with metabolism of later intermediary sterols to some extent. Compared with serum sterols, lanosterols were enriched in biliary and fecal sterols up to 10–20 times. Fecal lanosterol output increased from 12 to 247 mg/day, and comprised over 20% fecal steroids of endogenous origin. Bile acid synthesis was significantly decreased, the proportion of chenodeoxycholic acid being markedly reduced in both biliary and fecal bile acids. Cholesterol absorption appeared to decrease yet fecal neutral sterol output and cholesterol synthesis were unchanged and the overall sterol synthesis was increased. It thus appears that ketoconazole inhibits cholesterol elimination as bile acids. However, by blocking 14α-demethylation, it results in effective drainage of sterol nucleus as lanosterols into bile and feces, which, in turn, is associated with a marked reduction in low density lipoprotein (LDL) cholesterol level probably through activation of hepatic LDL apoB receptors.—**Miettinen, T. A.** Cholesterol metabolism during ketoconazole treatment in man. *J. Lipid Res.* 1988. **29: 43**–51.

Supplementary key words serum cholesterol • cholesterol synthesis • methyl sterols • lanosterol • lathosterol • bile acids • biliary sterols • fecal steroids • LDL cholesterol

Ketoconazole is an effective antifungal agent (1), which inhibits cytochrome P-450-dependent enzymes (2). These enzymes are involved in the demethylation of lanosterol (3) and hydroxylation during steroid hormone synthesis (2, 4, 5). Consequently, disturbances can be recorded in cholesterol and steroid hormone metabolism to the extent that large doses of ketoconazole have been used for hormonal orchiectomy in prostate cancer (6) and for the treatment of conditions with adrenal or gonadal hormone overproduction (7–9). Large doses of the drug also effectively lower the serum cholesterol level (10) because it interferes with cholesterol synthesis mainly by inhibiting 14α-demethylation of lanosterol (11) so that lanosterol and dihydrolanosterol accumulate in the serum (12). The question now arises whether ketoconazole could also be used for the treatment of hypercholesterolemia or whether the side effects, e.g., accumulation of lanosterol and dihydrolanosterol, adrenal insufficiency, male hypogonadism, and liver damage, are severe enough to exclude its hypolipidemic use. The major purpose of the present study was to explore cholesterol metabolism and accumulation of cholesterol intermediates in patients treated for prostate cancer with large doses of ketoconazole.

MATERIALS AND METHODS

Patients

Five patients (**Table 1**) with metastasized prostate cancer were treated with 1.2 g/day of ketoconazole. They all were in a good nutritional condition and tolerated the treatment well without detectable side effects. However, despite a marked decrease in the serum testosterone levels of patients 2 (from 17.1 to 1.6 nmol/l) and 3 (1.0 to 0.4 nmol/l), the treatment had to be discontinued after 34 and 14 days, respectively, because of worsening bone pain. It was replaced by another treatment schedule. Three of

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; VLDL, LDL, and HDL, very low density, low density, and high density lipoprotein; BMI, body mass index; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; lanosterol, 14α,4α,4β-trimethyl-5α-cholest-8,24-dien-3β-ol; $\Delta^{8,24}$-dimethylsterol, 4α,4β-dimethyl-5α-cholest-8(9),24-dien-3β-ol; Δ^8-dimethylsterol, 4α,4β-dimethyl-5α-cholest-8(9)-en-3β-ol; Δ^8-methostenol, 4α-methyl-5α-cholest-8(9)-en-3β-ol; methostenol, 4α-methyl-5α-cholest-7-en-3β-ol; Δ^8-cholestenol, 5α-cholest-8(9)-en-3β-ol; lathosterol, 5α-cholest-7-en-3β-ol; desmosterol, cholest-5,24-dien-3β-ol; KC, ketoconazole; GLC, gas–liquid chromatography; TLC, thin- layer chromatography.

TABLE 1. Clinical data on patients with prostate cancer

Case No.	Age	Weight	Height	BMI	ASAT[a]	ALAT[a]	Orchiectomy	Estrogen Treatment
	yr	*kg*	*m*	*kg/m²*	*IU/ml*			
1	66	70	1.71	23.9	19	5	yes	earlier
		68[b]			27[b]	9[b]		
2	66	80	1.71	30.8	53	34	no	none
		81			21	18		
3	65	90	1.79	28.1	82	100	yes	none
		90			149	254		
4	73	86	1.81	26.3	78	138	no	earlier;
		89			28	22		current
5	76	60	1.64	22.3	37	14	yes	earlier;
		59			40	20		current

[a] Normal values; ASAT and ALAT < 40 IU/ml.
[b] Second values were obtained at the end of the ketoconazole treatment.

the patients had earlier been orchiectomized with some effect on metastases. Cases 4 and 5 also used estrogens during the ketoconazole treatment, while estrogen treatment had been discontinued earlier in patient 1.

Studies

The patients were hospitalized for the start of the treatment and up to three times during the treatment. They consumed the standard hospital food, with cholesterol intake of 300 mg/day. Cholesterol absorption, biliary, and fecal steroid measurements; serum cholesterol, triglyceride, and lipoprotein analysis; and quantitation of cholesterol precursors in serum and bile were performed before and during the ketoconazole treatment. The control studies were performed in patient 1 after discontinuation of the ketoconazole treatment. Cholesterol absorption (13) and fecal steroid output were measured in patients 1–4 from stools collected for 3 days after administration of a capsule (mixture of 200 mg of Cr_2O_3, [^{14}C]cholesterol, 0.24 μCi, and [^{3}H]β-sitosterol, 0.48 μCi) with each major meal three times a day for 7 days. Duodenal bile samples were obtained from patients 1 and 4 after intravenous administration of cholecystokinin by duodenal intubation during the control and treatment periods. The effect of cholestyramine (8–12 g/day) during the ketoconazole treatment was studied in patient 5; stool collections and analysis were omitted. The study protocol and studies were accepted by the ethical committee and informed consent was obtained from every patient.

Determinations

Serum total cholesterol and triglycerides, and VLDL, LDL, and HDL cholesterol and triglycerides were determined as described in the Manual for Laboratory Operation of the Lipid Research Clinics Program (14). From two to four measurements were made during both the control and treatment periods.

Cholesterol precursors and other non-cholesterol sterols, cholestanol and plant sterols, campesterol and sitosterol, were quantitated by gas-liquid chromatography (GLC) on a capillary column after the free and esterified sterols had been separated by thin-layer chromatography (TLC) mainly as described earlier for serum and bile (15, 16). Briefly, the chloroform–methanol extract of serum lipids is applied to the TLC plate and developed for the full length with heptane–toluene 9:1 to separate squalene (R_f, 0.78). The plate is redeveloped with ethyl ether–heptane 55:45 and four zones are separated in increasing order of mobility: *1*) free cholesterol fraction (R_f, 0.29); *2*) invisible fraction corresponding to the mobility of coprostanol (R_f, 0.38) and including free monomethylsterols and dimethylsterols; *3*) remaining methyl sterol area including dimethylsterols, and lanosterol and dihydrolanosterol (R_f, 0.45); and *4*) esterified sterols (R_f, 0.91). The ester fraction is saponified and separated by TLC into fractions 1–3 described above. The free and esterified methyl sterols must be divided into two parts (zones 2 and 3) for the quantitation of methostenol (present in TLC zone 2) and dihydrolanosterol (present in TLC zone 3) separately because they have almost identical retention times in the GLC method used. The procedure quantitates the following free and esterified sterols. Cholesterol fraction: cholesterol, cholestanol, Δ^8-cholestenol, desmosterol, lathosterol, campesterol, and β-sitosterol; methylsterol fractions: methostenol, Δ^8-methostenol, Δ^8-dimethyl (4α4β) sterol, $\Delta^{8,24}$-dimethylsterol, dihydrolanosterol, and lanosterol.

In patient 5 the major total (sum of free and esterified) non-cholesterol sterols were analyzed directly from non-saponifiable serum lipids by GLC, only the first serum sample being separated by TLC for reliable quantitation of dihydrolanosterol. Thus, total cholestanol, Δ^8-cholestenol, desmosterol, lathosterol, dihydrolanosterol, lanosterol, campesterol, and β-sitosterol were quantitated from other serum samples directly from the non-saponifiable material. All the values for the non-cholesterol sterols are expressed in terms of mmol/mol of cholesterol in order to relate the sterol contents within the

lipoproteins or bile to cholesterol (free, ester, or total).

Biliary squalene and sterols were determined by GLC from the nonsaponifiable lipids. Biliary bile acids were also quantitated by GLC (17) and phospholipids by the method of Bartlett (18).

Fecal steroids were quantitated by GLC on an SE-30 capillary column (17). During the ketoconazole treatment two large peaks with the retention times of dihydrolanosterol and lanosterol appeared in the GLC analyses. Mass spectrometric analysis gave a fragmentation pattern identical to the authentic lanosterol-dihydrolanosterol standard. The same fragmentation was also obtained from the respective peaks in the serum and biliary sterols and also from the small respective peaks found in GLC analyses of the fecal sterols under basal conditions.

RESULTS

Serum total lipids and lipoproteins

Ketoconazole had no effect on serum triglycerides, but the mean reduction of total serum cholesterol was 27% and that of LDL cholesterol was 41% **(Table 2)**. Apoprotein B of LDL was decreased by 32%, and the apoB/LDL cholesterol ratio tended to increase. The levels of HDL and VLDL cholesterol were not affected by ketoconazole.

Cholesterol precursors in serum

Free sterols. The means of concentrations of cholesterol precursor and other sterols in the sera of patients 1–4 are given in **Table 3** and for patients 1 and 4 in **Table 4**. Serum squalene increased inconsistently but the pattern of the serum cholesterol precursors changed dramatically with ketoconazole. A major change took place in the free lanosterol and dihydrolanosterol contents which were increased about 30 times and 250 times, respectively, yet they comprised only 3% of the total unesterified serum sterols. The contents of other unesterified precursor sterols increased much less but the sterols with the double bond at C-8 (Δ^8-dimethylsterols, Δ^8-methostenol and Δ^8-cholestenol) exhibited a fourfold to sixfold increase over the control level. On the other hand, the increases in the Δ^{24} sterols ($\Delta^{8,24}$-dimethylsterol and desmosterol) were inconsistent, and accumulation of the Δ^7 sterols (methostenol, and lathosterol) was relatively small.

Esterified sterols. Since the methyl sterols, in contrast to the demethylated precursor sterols, are not esterified in serum (19), their ester contents were largely unchanged. However, the huge increase in free lanosterol and dihydrolanosterol was associated with small but consistent increases in their ester levels as well. The degree of esterification of lanosterol and dihydrolanosterol decreased from 18% and 35% to 9% and 8%, respectively. The sum of the total serum concentration of the two lanosterols was 39 μmol/l (1.5 mg/dl) and comprised 1.1% of total serum cholesterol. Of the demethylated esterified precursors, Δ^8-cholestenol exhibited the highest relative increase; the desmosterol content was unchanged.

Other sterols

In terms of mmol/mol of cholesterol, the serum contents of free campesterol and β-sitosterol and also of esterified cholestanol were significantly increased by the ketoconazole treatment. The changes in the total contents of plant sterols and cholestanol were not significant, however.

Biliary lipids

Biliary and serum squalene and sterol composition of patients 1 and 4, whose bile and serum were sampled simultaneously for comparison, are presented in Table 4 during the control and ketoconazole treatment periods.

TABLE 2. Effects of ketoconazole treatment (KC) on serum lipids and lipoproteins in patients with prostate cancer

Case No.	Treatment	Serum Cholesterol					Triglycerides	
		Total	VLDL	HDL	LDL	LDL-ApoB	Total	VLDL
		mmol/l				*mg/dl*	*mmol/l*	
1	none	6.89	0.62	1.39	4.88	128	1.06	0.46
	KC	4.56	0.55	1.66	2.17	76	1.18	0.59
2	none	5.80	0.60	0.95	4.24	113	1.46	0.80
	KC	4.18	0.46	0.90	2.81	80	2.00	0.57
3	none	6.20	0.32	1.30	4.54	125	1.37	0.71
	KC	4.40	0.32	1.21	2.83	87	1.23	0.51
4	none	5.92	0.56	2.89	2.40	93	2.40	1.12
	KC	4.95	0.41	2.74	1.75	68	1.81	1.19
Mean ± SEM	none	6.20 ± 0.24	0.53 ± 0.07	1.63 ± 0.43	4.02 ± 0.55	115 ± 8	1.57 ± 0.29	0.77 ± 0.14
Mean ± SEM	KC	4.52 ± 0.16[a]	0.44 ± 0.05	1.63 ± 0.40	2.39 ± 0.26[a]	78 ± 4[a]	1.56 ± 0.20	0.72 ± 0.16

Values are means of two to four determinations.
[a]Statistically significant ($P < 0.05$ or less) change compared to no treatment.

TABLE 3. Effects of ketoconazole (KC) on free and esterified serum cholesterol, cholesterol precursors, cholestanol, and plant sterols[a]

Compound	Control		Ketoconazole		KC/Control	
	Free	Ester	Free	Ester	Free	Ester
			mmol/l			
Cholesterol	1.5 ± 0.1	3.2 ± 0.1	1.2 ± 0.1[b]	2.3 ± 0.1[b]	0.8	0.7
			mmol/mol of cholesterol · 10^{-2}			
Squalene	17 ± 5		24 ± 4		1.4	
Lanosterol	30 ± 7	3 ± 1	977 ± 74[b]	51 ± 16[b]	32.6	17.0
Dihydrolanosterol	8 ± 3	2 ± 1	988 ± 159[b]	93 ± 20[b]	248.5	46.5
$\Delta^{8,24}$-Dimethylsterol	27 ± 7	2 ± 0	35 ± 8	4 ± 1	1.3	2.0
Δ^{8}-Dimethylsterol	22 ± 5	2 ± 1	133 ± 38[b]	6 ± 1	6.0	3.0
Δ^{8}-Methostenol	16 ± 5	8 ± 2	61 ± 19[b]	14 ± 1	3.8	1.8
Methostenol	20 ± 5	12 ± 3	38 ± 11[b]	12 ± 1	1.9	1.0
Δ^{8}-Cholestenol	12 ± 4	11 ± 2	61 ± 8[b]	39 ± 6[b]	5.1	3.5
Lathosterol	231 ± 58	85 ± 16	330 ± 83[b]	125 ± 24[b]	1.4	1.5
Desmosterol	35 ± 5	40 ± 5	61 ± 14	58 ± 9	1.7	1.5
Cholestanol	118 ± 31	83 ± 18	130 ± 12	115 ± 15[b]	1.1	1.4
Campesterol	108 ± 15	112 ± 34	156 ± 15[b]	133 ± 21	1.4	1.2
β-Sitosterol	93 ± 16	108 ± 26	123 ± 12[b]	127 ± 10	1.3	1.2

[a] All values are means ± SEM of four patients with prostate cancer.
[a] Statistically significant ($P < 0.05$ or less) change.

As shown earlier (16, 20), lanosterol is concentrated more effectively in bile under basal conditions than are other methylsterols. During the ketoconazole treatment the two lanosterols were so effectively cleared into the bile that the sum of the two increased from the base line values of 1.5 and 0.4 to 42 and 24 mg/100 mg of cholesterol in patients 1 and 4, respectively. The latter values are about ten times higher than in the serum (2.5–3.0 mg/100 mg of free cholesterol) in the two patients.

Like the serum values, Δ^{8}-dimethylsterol, Δ^{8}-methostenol, and Δ^{8}-cholestenol increased moderately in the bile during the ketokonazole treatment, whereas the contents of other sterols changed inconsistently.

Table 5 shows the molar composition of biliary sterols, phospholipids, and bile acids in patients on and off ketoconazole. The molar percentage of lanosterols increased from about 0.1 to 2.7 and 2.0 in the two patients. Dramatic changes are also seen in the bile acid pattern. Thus,

TABLE 4. Effects of ketoconazole on serum free and biliary cholesterol, cholesterol precursors, cholestanol, and plant sterols in two patients with prostate cancer

Compound	Case 1				Case 4			
	Control		Ketoconazole		Control		Ketoconazole	
	Serum	Bile	Serum	Bile	Serum	Bile	Serum	Bile
				mmol/l				
Cholesterol	1.5	0.4	1.2	0.9	1.5	1.7	1.2	1.6
				mmol/mol of cholesterol · 10^{-2}				
Squalene	27	260	19	194	13	53	15	35
Lanosterol	58	1346	903	20194	12	327	900	10485
Dihydrolanosterol	13	208	1656	21984	3	41	2077	13087
$\Delta^{8,24}$-Dimethylsterol	29	209	33	84	13	79	15	70
Δ^{8}-Dimethylsterol	37	187	85	376	7	110	57	309
Δ^{8}-Methostenol	26	267	38	305	8	80	28	187
Methostenol	27	230	46	91	8	99	16	66
Δ^{8}-Cholestenol	12	55	70	271	8	23	50	49
Lathosterol	243	638	327	715	266	875	278	844
Desmosterol	33	46	98	103	38	127	36	67
Cholestanol	60	562	99	500	136	832	139	835
Campesterol	130	552	157	409	65	186	116	344
β-Sitosterol	151	630	134	410	47	1322	88	1586

Serum and bile were sampled simultaneously.

TABLE 5. Biliary lipid composition on and off ketoconazole treatment

Compound	Case 1		Case 4	
	On	Off	On	Off
Cholesterol, molar %	8.5	10.2	6.2	4.3
Lanosterols, molar %	2.0	<0.1	2.7	0.1
Phospholipids, molar %	3.5	5.8	4.6	2.0
Bile acids, molar %	86.0	84.0	86.6	93.7
Cholic acid, %	63	74	61	40
Chenodeoxycholic acid, %	2	11	3	24
Deoxycholic acid, %	35	15	36	36

the proportion of chenodeoxycholic acid decreased from 11% and 24% to 2% and 3% in the two cases, respectively.

Cholesterol absorption and fecal steroids

Table 6 presents data on fecal sterols, bile acids, and cholesterol absorption during the control and ketoconazole periods. Fecal excretion of neutral sterols of cholesterol origin was unaffected by ketoconazole despite the finding that the fraction of cholesterol absorbed was reduced in all three cases in which it was studied. Proportions of coprostanol and coprostanone were unchanged (data not shown), indicating that bacterial action was unaffected. Dietary cholesterol, fractional cholesterol absorption, and fecal sterols allowed calculation of total and endogenous cholesterol fluxes into the intestinal lumen and absolute cholesterol absorption during the two treatment periods of the three subjects. The mean total flux during the control period was 1342 mg/day and during the ketoconazole period 1166 mg/day; the respective endogenous influxes were 1042 and 866 mg/day. Thus, the total absorption decreased from 684 to 443 mg/day (individual decrements varied from 187 to 327 mg/day), but the fecal output of endogenous neutral sterols of cholesterol origin was unchanged (511 vs. 537 mg/day).

During the ketoconazole treatment two major peaks with the retention times and mass spectrometric fragmentations of lanosterol and dihydrolanosterol appeared in the GLC analysis of the fecal neutral sterols. The sum of the two lanosterols (see Table 6) increased from 12 to 247 mg/day and, when included in the fecal neutral sterols of cholesterol origin, the grand total fecal output of neutral sterols was increased by ketoconazole from 776 mg/day to 1049 mg/day ($+273 \pm 54$ mg/day). During the treatment the lanosterol content in feces was 31 mg/100 mg of neutral sterols and 45 mg/100 mg of endogenous neutral sterols of cholesterol origin. The corresponding value in serum was 2 mg/100 mg of free cholesterol and in bile 33 mg/100 mg of biliary cholesterol.

Ketoconazole reduced mean fecal bile acid excretion from 258 to 193 mg/day (Table 6). Proportions of primary bile acids were unchanged—a sign of unaltered bacterial action. However, a closer inspection of the GLC analyses revealed that identifiable bile acids of chenodeoxycholic acid origin (chenodeoxycholic acid, lithocholic acid, isolithocholic acid, and ursodeoxycholic acid) virtually disappeared during the treatment. In fact, their absolute amount decreased from 57 to 12 mg/day.

The fecal steroid data indicated that cholesterol elimination was unchanged during the ketoconazole treatment. However, addition of the lanosterols to the steroid data revealed that the grand total steroid elimination was increased by 205 ± 58 mg/day (from 1033 to 1238 mg/day), suggesting that the drug actually enhanced overall sterol synthesis in these patients.

Ketoconazole combined with cholestyramine

To study the effectiveness of ketoconazole during enhanced cholesterol synthesis, cholestyramine was administered to a patient treated with ketoconazole as shown in **Fig. 1**. The combination treatment further reduced the serum level of total cholesterol by 40%, the

TABLE 6. Effects of ketoconazole (KC) on fecal steroids in patients with prostate cancer

Case No.	Treatment	Fecal Steroids, mg/day							
		Bile Acids		Neutral Sterols	Total Steroids	Dihydrolanosterol	Lanosterol	Grand Total	Absorption %
1	none	291	(15.0)[a]	809	1100	5	12	1117	47
	KC	254	(4.4)	817	1071	143	112	1326	39
2	none	246	(31.4)	555	801	1	4	806	54
	KC	187	(3.9)	727	914	138	128	1170	39
3	none	240	(30.0)	1083	1323	3	11	1337	
	KC	131	(12.4)	1051	1182	119	134	1435	
4	none	245	(11.2)	608	853	2	8	863	53
	KC	199	(3.8)	611	810	130	82	1022	37
Mean ± SEM	none	258 ± 11	(21.9 ± 5.1)	764 ± 120	1021 ± 122	3 ± 1	9 ± 2	1033 ± 124	51 ± 2
Mean ± SEM	KC	193 ± 25[b]	(6.1 ± 2.1)[b]	802 ± 93	994 ± 82	133 ± 5[b]	114 ± 12[b]	1238 ± 90[b]	38 ± 1[b]

Bile acids + neutral sterols = total steroids; total steroids + dihydrolanosterol + lanosterol = grand total.
[a] Figures in parentheses are percentages of fecal bile acids due to sum of lithocholic, isolithocholic, chenodeoxycholic, and ursodeoxycholic acids.
[b] $P < 0.05$ or less.

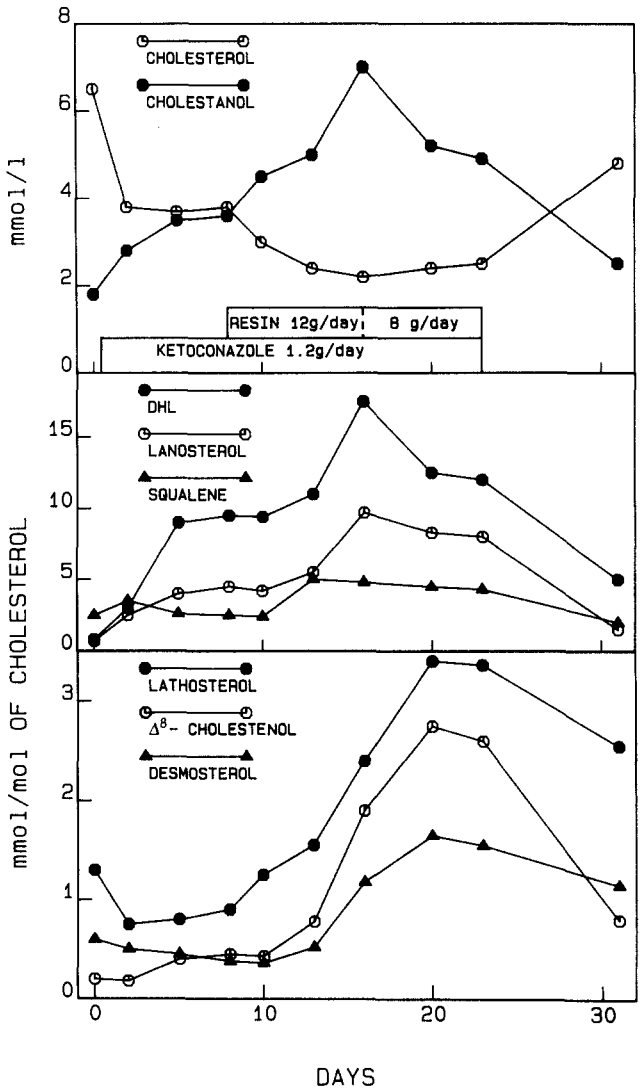

Fig. 1. Effects of ketoconazole and cholestyramine (resin) on serum total sterols in a patient with prostate cancer. Cholestanol in terms of mmol/mol of cholesterol; DHL, dihydrolanosterol.

total reduction being 65%. The cholestanol content increased up to fourfold with the combined treatment. As expected, ketoconazole dramatically increased the levels of lanosterol and dihydrolanosterol, the contents being almost doubled by the addition of cholestyramine. Squalene responded slightly. Lathosterol and desmosterol responded markedly (4–5 times) only to the cholestyramine addition, whereas the Δ^8-cholestenol level was doubled by ketoconazole and then further increased fivefold by cholestyramine.

DISCUSSION

The present study revealed two major changes in cholesterol metabolism during ketoconazole treatment in man in vivo: inhibition of lanosterol-14-demethylation and subsequent accumulation of lanosterols in serum and, especially, secretion in bile and feces. Secondly, there was moderate inhibition of overall bile acid synthesis, resulting in a marked reduction in chenodeoxycholic acid or its products in the bile and feces. As shown earlier (6, 10), the serum cholesterol level was clearly reduced owing to a decrease in the LDL cholesterol concentration. No consistent change was recorded in serum levels of total or VLDL triglycerides, even though long-term ketoconazole treatment has been reported to increase serum triglyceride contents (21).

Squalene levels were not consistently changed by the ketoconazole treatment, indicating that the drug hardly interfered with the conversion of squalene to lanosterol. Recent in vitro studies have shown that large enough amounts of ketoconazole can also inhibit squalene cyclization in human fibroblast cultures (22).

Of the processes involved in the conversion of lanosterol to cholesterol (**Fig. 2**), 14α-demethylation is cytochrome P-450-dependent (3), whereas 4α-demethylation and Δ^5-desaturation of Δ^7-cholestenol are catalyzed by mixed function oxidase without direct cytochrome P-450 re-

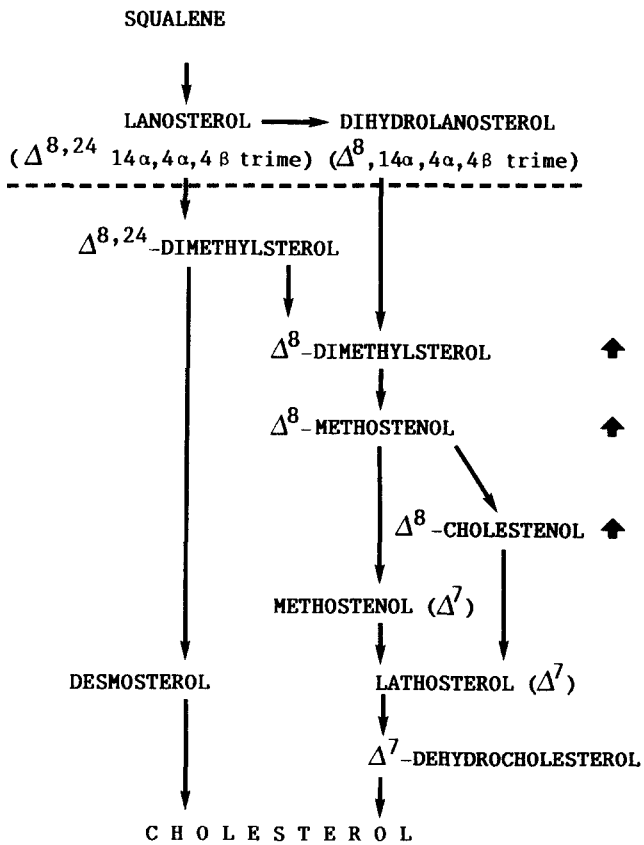

Fig. 2. Scheme of cholesterol synthesis from squalene through side chain unsaturated and saturated pathways. Trime, trimethylsterol; the horizontal dashed line illustrates main blocking of cholesterol synthesis by ketoconazole; the arrows (⬆) indicate postlanosterol precursor sterols increased by ketoconazole in serum and bile.

quirement (23), and microsomal Δ^8-isomerase catalyzes the conversion of Δ^8 to Δ^7 (24). Since ketoconazole effectively inhibits cytochrome P-450-dependent mixed-function oxidases, it interferes with the further conversion of lanosterol and explains our earlier (12, 25) and present findings on the accumulation of the two lanosterols. A 46% increase in serum lanosterol has recently been reported by Kraemer and Pont (10), and ketoconazole is known to block the conversion of lanosterol to cholesterol in cultured human fibroblasts (10, 22). In in vitro systems, ketoconazole-induced accumulation of lanosterol or its oxyderivatives may be a primary regulator of HMG-CoA reductase (22, 26). Obviously ketoconazole has no effect on saturation of Δ^{24} in vivo because the increase in lanosterol was smaller than that of dihydrolanosterol and the amounts of desmosterol pathway sterols were not consistently increased. In fact, inhibited conversion of lanosterol to $\Delta^{8,24}$-dimethylsterol may favor dihydrolanosterol formation and its further metabolism through the side-chain saturated pathway to cholesterol. The consistent four- to sixfold mean increase in other Δ^8-sterol contents, but not in the Δ^7-sterol contents, may point to inhibition of Δ^8-isomerase. Whether 4α-methyl oxidation is also affected is not known because methostenol contents increased inconsistently.

In view of the marked LDL cholesterol reduction, the accumulation of lanosterols in serum was surprisingly small in absolute terms. Thus, even though the relative increase was up to 250-fold, the mean serum concentration of total lanosterols was only 1.5 mg/dl during the ketoconazole treatment. The few sterol analyses of red cells showed lanosterol contents comparable to those of the serum free sterols, suggesting that free lanosterols are actually incorporated in cell membranes. Tissue deposits of noncholesterol sterols can occur at relatively low serum concentrations in phytosterolemia and especially in cerebrotendinous xanthomatosis (27). Thus, the possibility that lanosterols cause harmful tissue deposits when ketoconazole is used for long periods is not excluded. However, in bile acid malabsorption of ileal dysfunction, e.g., ileal resection or ileal bypass, a compensatory increase in cholesterol synthesis increases methyl sterols (including lanosterol) in serum lipoproteins by up to 20-fold (28), yet no harmful tissue deposits of sterols have been discovered, and tissue lanosterol levels are surprisingly low (29).

Our earlier studies have shown that lanosterol is enriched more markedly in bile than are other noncholesterol sterols (16, 20) and is found in gallstones only in negligible amounts (30). Preferential biliary secretion of lanosterols could be the reason why their serum contents remained relatively low during ketoconazole therapy. In fact, compared with the free sterols in serum, the lanosterol contents in the bile sterols were 6–22 and 14–27 times higher on and off ketoconazole, respectively. This would mean that at least hepatic lanosterols, but also the lanosterols possibly released from extrahepatic tissues in serum, would be effectively cleared into the bile. Owing to good biliary solubility, lanosterol may not contribute to gallstone formation (30), even though it clearly increases the molar percentage of biliary sterols during ketoconazole treatment (Table 3).

Surprisingly large amounts of lanosterols were excreted in feces during the ketoconazole treatment, indicating that lanosterols secreted in bile were not absorbed or that lanosterols were secreted by the intestinal mucosa. Lanosterol absorption is not known in man. The biliary contents of lanosterols were 42.1 and 23.5 mg/100 mg of cholesterol in cases 1 and 4, respectively. In feces, the respective values were 40.2 and 50.2 mg/100 mg of endogenous neutral sterols, suggesting that, in case 1, lanosterols were absorbed in proportion to cholesterol.

Preliminary results of this study indicated that chenodeoxycholic acid synthesis was reduced by ketoconazole (25). However, a recent study clearly indicated that in cultured rat and human hepatocytes, ketoconazole blocked bile acid synthesis by inhibiting cholesterol-7α-hydroxylase but not bile acid synthesis from 7α-OH cholesterol; there was no evidence that 12α-hydroxylation was inhibited (31). The present in vivo studies revealed that ketoconazole inhibited 7α-hydroxylase slightly but significantly because the total bile acid production was lowered. However, the amounts of biliary and fecal chenodeoxycholic acid were markedly reduced, indicating that the 12α-hydroxylase was down-regulated. Since the serum cholestanol content increased inconsistently (see Fig. 1 and Table 3), the mechanism of reduced chenodeoxycholic acid synthesis may differ from the mechanisms presented for cholestanolosis (27, 32, 33). Since several cytochrome P-450-dependent enzymes are involved in bile acid synthesis (34), ketoconazole in vivo may interfere not only with 7α-hydroxylase but also with other hydroxylations in bile acid formation.

Reduced bile acid synthesis, especially that of chenodeoxycholic acid, may have contributed to decreased cholesterol absorption via impaired micelle formation. Whatever the mechanism, reduced return of absorbed cholesterol to the liver and blocking of synthesis at the lanosterol step obviously depleted hepatocyte cholesterol. According to the current concept of cholesterol traffic (35), this, in turn, up-regulated hepatic LDL apoB receptors and resulted in LDL cholesterol reduction. In fact, preliminary LDL apoB turnover studies have suggested enhanced LDL catabolism during the ketoconazole treatment (25). Is overall cholesterol synthesis then inhibited by ketoconazole? In vitro studies in closed systems have revealed that synthesis is impaired (22, 26). The cholesterol balance data of the present study indicated that this is not consistently the case. Similar results have been obtained with mevinolin, i.e., an inconsistent net

decrease in fecal elimination of cholesterol during mevinolin-induced inhibition of synthesis and reduction of serum cholesterol levels (36). Inclusion of lanosterols in the sterol balance data in fact indicated improved overall sterol synthesis during ketoconazole treatment. Provided the high biliary lanosterol contents indicate that fecal lanosterols originate mainly from the liver and negligibly from the villous cells, this would mean stimulated HMG-CoA reductase activity and enhanced acetate flow to sterol synthesis in the liver. HMG-CoA reductase activity has been shown to be inhibited by low concentrations of ketoconazole, while higher concentrations appear to activate the enzyme in intestinal epithelial cell cultures (37). It can be inferred that hepatic cholesterol deficiency and the subsequent LDL apoB receptor activation and the reduction in serum cholesterol become more marked when the conversion of cholesterol to bile acids is stimulated with cholestyramine simultaneously with the ketoconazole treatment. Fig. 1 actually demonstrates the dramatic fall in the serum cholesterol level with the combination treatment but shows a surprisingly small further increase in lanosterol concentrations and a typical marked cholestyramine-induced increase in demethylated cholesterol precursor sterols as a sign of enhanced cholesterol synthesis (38).

The findings of the present study indicate that, by blocking 14α-demethylation of cholesterol synthesis, ketoconazole effectively drains sterols from the body into bile and feces mainly in the form of lanosterols, and results in an effective reduction in the serum LDL cholesterol level. Moreover, since long-term antifungal treatments have not revealed any serious sterol metabolism-related side effects, the use of ketoconazole to lower the serum cholesterol of hypercholesterolemic patients is worth testing. In fact, an agent that would specifically inhibit 14α-demethylation without interfering with steroid hormone production could be a useful adjunct to existing drugs for the treatment of hypercholesterolemic subjects.

Supported by grants from the Sigrid Juselius Foundation and the Finnish Academy for Medical Research. The clinical and secretarial assistance of Anja Salolainen, Eeva Gustafsson, and Marja Aarnio is acknowledged.

Manuscript received 1 May 1987 and in revised form 22 July 1987.

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