Tissue distribution of cholesterol and 24-dehydrocholesterol during chronic triparanol therapy

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SUMMARY The tissues of five patients who died following 4–31 months of continuous oral triparanol therapy have been analyzed for sterol content by gas–liquid chromatographic techniques. Desmosterol was present in variable amounts in all tissues examined, with the exception of nervous tissue, but in none of the tissues did desmosterol constitute a larger fraction of total sterols than that observed in the blood. No evidence of marked adrenal cholesterol depletion was apparent. Small but significant amounts of desmosterol were found in both relatively normal and atherosclerotic blood vessels. No preferential accumulation of desmosterol as compared with cholesterol was apparent in either the blood vessels or in any of the body tissues examined.

KEY WORDS desmosterol · cholesterol · metabolism · man · triparanol · gas–liquid chromatography · tissue sterols

THE WIDESPREAD USE of triparanol (1-[*p*-(β-diethylaminoethoxy)-phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl)ethanol) as an inhibitor of cholesterol biosynthesis and the demonstration by Steinberg and Avigan (1) that triparanol blocks the conversion of 24-dehydrocholesterol (desmosterol) to cholesterol have served to create interest in the effects of triparanol on the metabolism of both cholestrol and desmosterol in man. Previous studies in man have indicated that chronic triparanol therapy is associated with an accumulation of desmosterol in the plasma; total plasma sterols and the apparent sterol miscible pool appear to be depressed by the compound (2, 3) Marked similarities in the metabolism as well as in the chemical structures of cholesterol and desmosterol have been suggested by the work of Goodman, Avigan, and Wilson (4) who have found that desmosterol can be esterified at rates comparable to that of cholesterol and can serve as precursors for bile acid and steroid materials. However, some differences in their metabolism have also been suggested from the observations that the rate of excretion of desmosterol in the bile is more rapid than that of cholesterol (5) and that the rate of disappearance of radioactive desmosterol from the blood may be somewhat greater than that of cholesterol (4).

The present investigation has been carried out to study and compare the tissue distribution of desmosterol and cholesterol in human subjects treated with triparanol. The body tissues of five patients who died suddenly following chronic triparanol therapy have been analyzed for their sterol content by gas–liquid chromatography (GLC). The results indicate that desmosterol is present in measurable amounts in all tissues (excepting nervous tissue) of patients treated over prolonged periods with triparanol. The greatest percentage of desmosterol relative to total tissue sterols was generally observed in the plasma, with equal or lesser percentages found in the other body tissues.

MATERIAL AND METHODS

Five patients with coronary heart disease who had been on continuous triparanol therapy (250 mg/day) and who had died suddenly as the result of an acute myocardial

This is Paper III of *Body Cholesterol Metabolism in Man*. Part II, reference 6.

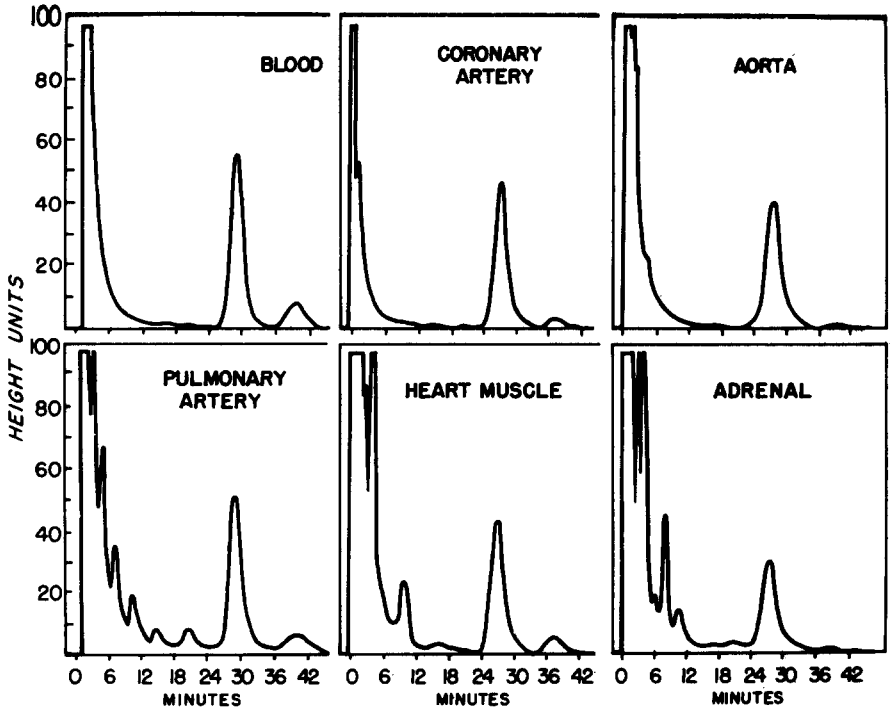

FIG. 1. GLC of sterols from tissues of patient 1, treated for 26 months with triparanol.

infarction were included in the study. In patients 1 and 2 (H.S. and D.L.), specimens of blood, liver, kidney, spleen, lung, adrenal gland, intestine, fat, muscle, brain, vena cava, pulmonary artery, coronary artery, aorta, and femoral artery were removed at autopsy 5 and 6 hr respectively after their death and were immediately frozen. During life, specimens of blood had been obtained from both patients at 2–4 week intervals throughout the period of triparanol therapy. In the three remaining patients, the tissues available for analysis had been fixed in formalin at the time of autopsy. All tissues were stored at −20° in sealed vials in a nitrogen atmosphere.

The intima was divided from the adventitia without attempting to separate out the media, and the degree of atherosclerosis was estimated grossly as previously described (6). All tissues were minced, and representative samples were dried to a constant weight. The tissue lipids were extracted according to the procedure of Folch et al. (7). Duplicate aliquots of the lipid extract were saponified with KOH, acidified, and then dried. The free sterols were converted to their methyl ethers using potassium tertiary butoxide and methyl iodide (8). The recovery of desmosterol on methylation ranged from 86 to 99% (average, 94%); that for cholesterol ranged from 89 to 100% with a mean of 96%. Recovery of desmosterol by the Folch procedure averaged 95% (91–98%) and that of cholesterol 98% (96–101%).

Following methylation, the sterol content of the tissue extract was analyzed by GLC according to a slight modification of the method of Clayton (8) using a modular chromatograph (Research Specialty Co.) with an ionization detector. Diethylene glycol succinate polyester (10% w/w) absorbed on Chromosorb W (60–80 mesh) was used as the column packing material. The vaporizing temperature was 295°, that of the outlet 260°, and of the detector 230°. Sterols from the tissues of patients 3–5 (A.L., A.D., and R.D.) were analyzed at a column temperature of 195° and those of patients 1 and 2 at a column temperature of 205°. The argon flow rate was 275 ml/min and its inlet pressure 35 psi. All determinations were carried out on the same 6 ft x 4 mm stainless steel column. The samples were injected in benzene through a rubber septum using a microliter hypodermic syringe. Each methylated aliquot of the tissue extract was analyzed in duplicate. The cholesterol and desmosterol contents were calculated by planimetric determinations of area and by measurement of peak heights. The areas and peak heights were compared with those obtained with standard mixtures of cholesterol and desmosterol which were analyzed daily. Variations in the amounts of cholesterol and desmosterol determined on duplicate analyses were not greater than 8%.

For the specimens of liver, adrenal gland, and brain, preliminary separation of the tissue sterols from other tissue lipids was made by thin-layer chromatography on Silica Gel G (Desaga) using petroleum ether–diethyl ether–acetic acid 90:10:1 (v/v/v) (9). The free sterol and sterol ester fractions were extracted from the adsorbent using benzene, and the combined extracts were saponified. The free sterols were converted to their methyl

ethers and analyzed as described above. The etherification procedure was always carried out in a nitrogen atmosphere in order to minimize the breakdown of desmosterol.

Aliquots of the tissue extracts of liver and thoracic aorta of patient 1, containing approximately 15 mg of free sterol, were also analyzed for their sterol content by silicic acid–Supercel column chromatography according to the method of Frantz (10). The sample was applied to a column 1 m long and 1.2 cm in diameter and 5-ml fractions of the eluting solvent were collected. Color was developed on 1-ml aliquots of each fraction by the Liebermann Burchard reaction (11) and the color was read at 620 mμ after 1.5 and 30 min. The remaining portions of the fractions were combined in groups of five tubes and analyzed by GLC as previously described.

RESULTS

The results of the tissue analyses in patients 1 and 2 are tabulated in Table 1. A summary of the results of the blood vessel analyses are listed separately in Table 2.

Representative gas chromatographic tracings of the tissue extracts in patient 1 are illustrated in Fig. 1. Under the conditions of these studies, cholesterol appeared first on the tracing, followed by desmosterol. The retention time of desmosterol varied between 1.4 and 1.5 times that of cholesterol. Complete separation of cholesterol and desmosterol was achieved in all instances. No tissue sterols other than cholesterol or desmosterol were apparent in any of the tracings.

With column chromatography of the sterols of both liver and aorta of patient 1, only two sterol peaks, both of "slow-acting" material (as determined by the Liebermann-Burchard reaction), could be identified. The earlier, major, component (fraction 71–90) had a retention time on GLC identical with that of cholesterol. The major component of the second peak (fractions 91–105) had the retention time by GLC of desmosterol. In addition, the initial segment of the second peak (tubes 91–95) also contained small amounts of sterol with a retention time equal to that of cholesterol.

Tissue Sterol Content (Table 1)

In patient 1, the blood desmosterol content at the time of death was 38.8 mg/100 ml, or 17% of the total blood sterols. During the 6 months before death, the plasma desmosterol ranged from 34 to 41 mg/100 ml (15–18% of total sterols). The total sterol level of the plasma prior to triparanol therapy averaged 332 mg/100 ml and during triparanol therapy 231 mg/100 ml. In the other body tissues, the desmosterol content varied widely from a maximum of 7.30 mg/g of dry tissue in the adrenal gland down to zero in the brain. In the spleen, heart muscle, and intestine, the desmosterol percentage of total sterols was similar to that found in the blood but was smaller in the other tissues.

Corresponding figures for patient 2 were: plasma desmosterol at death, 39.2 mg/100 ml (16% of total plasma sterols); for the 6 months before death, 36–40 mg/100 ml (15–17%); total plasma sterols before treatment, 398 mg/100 ml; after, 250 ml/100 ml. In the tissues, the same qualitative observations as for patient 1 were made.

Blood Vessels (Table 2)

The desmosterol content of the arterial intima varied from 0 to 5.77 mg/g of dry tissue, or 0–8% of total intimal sterols. In patients 1, 2, and 5, in whom both arterial and blood sterols were measured, the desmosterol content of

TABLE 1 DEMOSTEROL AND CHOLESTEROL CONTENT OF TISSUES AFTER CHRONIC TRIPARANOL THERAPY

Patient		Tissue										
		Blood	Liver	Spleen	Lung	Kidney	Adrenal	Striated Muscle	Heart Muscle	Intestine	Fat	Brain
1, male, 70 yr; triparanol 26 months	Desmosterol (*mg/g dry tissue*)	38.8*	1.15	3.34	1.15	1.11	7.30	0.27	1.09	0.67	0.28	0
	Cholesterol (*mg/g dry tissue*)	193.	11.9	17.6	18.1	14.8	119.	3.08	6.17	3.26	2.50	96.7
	Desmosterol / Cholesterol + Desmosterol	0.17	0.09	0.16	0.06	0.07	0.06	0.08	0.15	0.17	0.10	0
2, male, 60 yr; triparanol 31 months	Desmosterol (*m/g dry tissue*)	39.2*	2.53	3.04	2.28	1.93	4.21	0.45	1.65	0.88	0.04	0
	Cholesterol (*mg/g dry tissue*)	207.	25.1	19.2	19.0	15.5	100.3	4.12	8.36	5.40	0.94	132.
	Desmosterol / Cholesterol + Desmosterol	0.16	0.09	0.14	0.11	0.11	0.04	0.10	0.16	0.14	0.04	0

* = mg/100 ml.

the intima varied from 0 to 8% of total sterols and that of blood from 12 to 17%. In patient 3, in whom only the cardiac muscle sterol content was available for comparison, the desmosterol content of segments of thoracic aorta and coronary artery were 3 and 2% respectively, of total intimal sterols as compared to 9% in the cardiac muscle.

In the four instances in which different adjacent areas of intima were analyzed and compared, the percentage of total sterols represented by desmosterol was lower in severely atherosclerotic than in relatively normal segments. In three of the specimens the total content of desmosterol per gram of dry tissue was somewhat lower in the atherosclerotic areas than that observed in adjacent relatively normal segments; in the fourth, it was higher.

The desmosterol to total sterol ratios in segments of pulmonary artery and of inferior vena cava were greater than those in the systemic arteries and were almost equal to those in the plasma of the same patients.

DISCUSSION

This study indicates that desmosterol is present in significant amounts in the body tissues of all human patients who have been treated over prolonged periods with triparanol. The amount of desmosterol varied from one tissue to another and was not directly related to the total tissue sterol content. The fraction of total sterol represented by desmosterol was greatest in the blood, spleen, intestine, and heart muscle. The amount of desmosterol in all instances represented a relatively small fraction of total tissue sterols. Cholesterol still comprised more than 80% of the sterol content of all tissues examined even after 31 months of triparanol therapy.

The widely differing tissue ratios of desmosterol to cholesterol suggest that the body metabolism of these chemically similar compounds differs in certain respects. It has been shown (6) that the cholesterol in all human tissues outside the central nervous system is exchangeable with the plasma cholesterol. If cholesterol and desmosterol were biologically identical, similar ratios of the two sterols in all tissues constituting the body cholesterol miscible pool should eventually be attained. Actual rates of exchange of desmosterol between the plasma and tissues have not been studied in man but the studies of Goodman, Avigan, and Wilson (4) demonstrating that labeled desmosterol disappears from the plasma somewhat more rapidly than cholesterol suggest that differences may exist in the rate of exchange of desmosterol and cholesterol between the blood and tissues. Increased tissue turnover of desmosterol as compared to cholesterol could also

TABLE 2 DESMOSTEROL AND CHOLESTEROL CONTENT OF BLOOD VESSELS AFTER CHRONIC TRIPARANOL THERAPY

Subject (All Male)	Age	Triparanol	Tissue	Degree of Atherosclerosis (O–IV)	Desmosterol	Cholesterol	Desmosterol / Desmosterol + Cholesterol
	yr	*months*			*mg/g dry tissue*		
1	70	26	Blood	—	38.8*	193.*	0.17
			Vena Cava	0	0.53	3.52	0.13
			Pulmonary Artery	0	0.66	4.10	0.14
			Coronary Artery	II	3.50	41.3	0.08
				IV	2.39	66.7	0.03
			Thoracic Aorta	II	2.52	35.7	0.07
				IV	1.08	44.9	0.02
			Abdominal Aorta	IV	5.77	84.2	0.06
			Femoral Artery	III	2.11	39.8	0.05
2	60	31	Blood	—	39.2*	204.*	0.16
			Vena Cava	0	0.66	4.81	0.12
			Pulmonary Artery	0	0.33	2.63	0.11
			Coronary Artery	IV	2.74	58.8	0.04
			Thoracic Aorta	II	2.81	32.2	0.08
				IV	5.70	101.	0.05
			Abdominal Aorta	II	2.06	30.6	0.06
				IV	1.72	53.7	0.03
3	49	10	Heart Muscle	—	0.84	8.50	0.09
			Thoracic Aorta	III	2.03	64.3	0.03
			Coronary Artery	IV	1.80	85.2	0.02
4	62	4	Abdominal Aorta	IV	4.12	131.	0.03
5	58	6	Plasma	—	35.9*	263.*	0.12
			Liver	—	1.21	19.5	0.06
			Abdominal Aorta	IV	0	60.8	0

* = mg/100 ml.

contribute to the observed low desmosterol to total sterol fractions, and the finding that the rate of conversion of desmosterol to bile salts was significantly greater than that of cholesterol (5) could possibly explain the relatively low desmosterol content in the liver of these patients.

The adrenal cholesterol levels of these patients were at the lower limits of the range reported for individuals dying as a result of acute myocardial infarctions (12). The adrenal desmosterol content represented a relatively small fraction of total adrenal sterols. These findings are in contrast to those reported in rats, where large doses of triparanol have produced marked depletion of adrenal cholesterol and marked accumulation of adrenal desmosterol (13). The differences may be related to dosage, since the clinical doses of triparanol used in the present study were only a fraction of those administered to the experimental animals. The absence of a marked reduction in adrenal cholesterol content observed here, and the previous observations indicating that desmosterol as well as cholesterol can serve as substrate for steroid hormone formation, suggest that the reduced urinary excretion of corticosteroids observed clinically during triparanol therapy (3, 14) may not necessarily be related to a simple reduction in the total amount of sterol available as substrate for conversion to steroid hormones. In vitro studies indicate that triparanol is capable of blocking a number of enzyme systems other than those involved in the reduction of desmosterol to cholesterol (15) and the possibility of another effect of the drug, such as interference with the release of hormone from the adrenal or even with the peripheral utilization of corticosteroids by the tissues, cannot be excluded.

Since triparanol has been used for the treatment of hypercholesterolemia and vascular disease, the accumulation of desmosterol in the arterial intima has become of interest. The present studies are in agreement with recent reports in man and animals demonstrating the presence of desmosterol in the intima during triparanol therapy (16–18). Comparison of the rate of penetration into the arterial wall of desmosterol compared with that of cholesterol has not been carried out in man. Previous studies in this laboratory have suggested that complete equilibration of cholesterol between the plasma and normal arterial intima can occur within 4–7 months after the intravenous administration of cholesterol-C^{14} (6). In the present study, all of the arterial specimens had relatively low desmosterol:total sterol ratios (less than one-half of that in the blood). However, all vessels had moderate to severe degrees of atherosclerosis, and the low content of desmosterol in the intima may merely have been a reflection of the effects of atherosclerosis in delaying the equilibration of desmosterol in the plasma with that in the intima, just as has been demonstrated with cholesterol. The low desmosterol contents in the intima even after almost 3 yr of triparanol therapy do suggest that desmosterol does not accumulate preferentially over cholesterol in the vessel wall.

Cholesterol and desmosterol were the only tissue sterols that could be demonstrated by either GLC or column chromatography. The study does not, however, rule out the possibility that undetected amounts of other sterol materials may also have been present. 7-Dehydrocholesterol does not separate from desmosterol by either of the chromatographic methods employed and may have been overlooked as a separate entity (19). Likewise, Δ^8-cholestenol may have escaped detection in the cholesterol GLC peak (19). In addition, it is possible that highly polar sterols may not have appeared at all on the gas chromatogram. Possible alterations in sterol structure due to post-mortem changes or to the chemical methods employed represent still another potential cause for error. No human skin was available for analysis and it is unknown whether the sterol precursors of cholesterol described for the skin of triparanol-treated rats (19, 20) may have been present in these patients.

The absence of desmosterol in the brain of these patients is consistent with previous observations indicating that no significant exchange of cholesterol between the plasma and brain occurs in man (6). The results in man differ from those reported for young rats in which both cholesterol and desmosterol have been shown to enter into the brain in appreciable amounts from the plasma (21, 22).

The authors would like to express their thanks to Drs. R. B. Clayton and H. Wotiz for their advice concerning the chromatographic methods used in these studies and to Drs. W. Stokes and T. Blohm for their generous gifts of desmosterol. The authors also thank Dr. M. Halperin and Dr. J. Budnitz for making available tissues specimens from their patients.

These studies were supported in part by the Research Program Project Grant HE-07299, Public Health Grant HE-1536, and the U. A. Whitaker Fund.

Manuscript received June 1, 1964; accepted September 16, 1964.

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