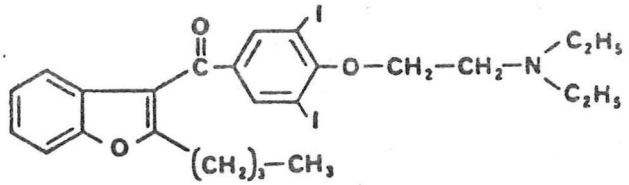
PHOSPHOLIPID FATTY LIVER

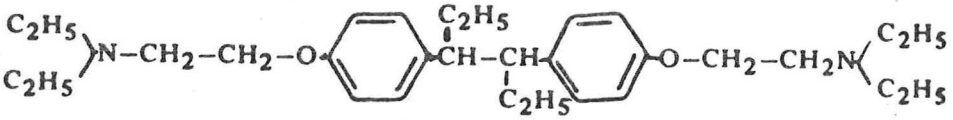
A TALE OF FOUR DRUGS

Burton Combes, M.D.

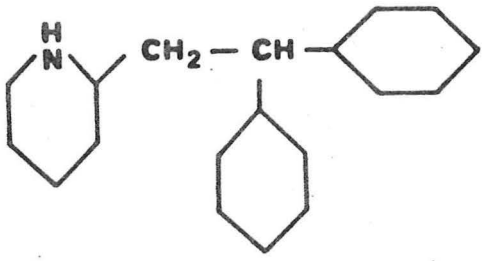
1. Amiodarone



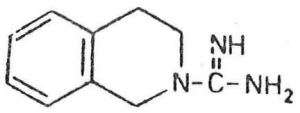
2. 4,4'-Diethylaminoethoxyhexestrol



3. Perhexiline maleate



4. Debrisoquine



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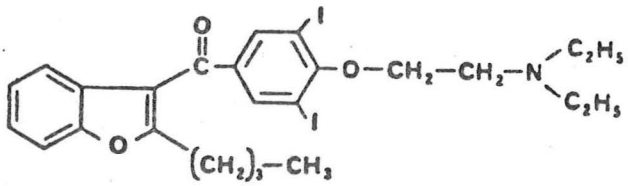


Case Report

A.J. Date of Birth 1.25.08

This white woman was admitted to hospital 4.8.83 with findings of an acute anterolateral myocardial infarct. Her course was complicated by episodes of PVCs, atrial fibrillation, and recurrent bouts of ventricular tacchycardia. The latter were not controlled by lidocaine, inderal, quinidine, or pronestyl. A temporary pacer was placed for override pacing and finally an AV sequential permanent pacemaker was inserted. Ventricular tacchycardia recurred while on pronestyl and the pacer.

Amiodarone was begun on 4.23.83 at a dose of 800 mg per day and pronestyl was continued. Subsequently she continued to have 100% paced rhythm and no evidence of ectopy. Pronestyl was gradually decreased, then stopped. By discharge on 5.14.83, amiodarone had been decreased to 200 mg t.i.d., and the AV sequential pacemaker turned down from an initial rate of 90 to 80 per minute. Subsequently, she did not experience symptomatic arrhythmias until her death in June 1984.

Several months after beginning amiodarone, she began to complain of profound weakness in her lower extremities. In February 1984 she experienced anorexia, nausea and epigastric discomfort. SGOT and SGPT were elevated for the first time to 182 and 112 units, respectively. With persistence of symptoms and the appearance of dark urine, the patient was hospitalized in June 1984. Amiodarone dosage had been decreased gradually during the symptomatic period.

She appeared chronically ill and mildly icteric. Liver span was 14 cm in the right midclavicular line. The spleen was not palpable. Modest ascites was detected. Serum bilirubin was 3.8 mg/dl, SGPT was 68, SGOT 147 units/liter (upper limit of normal was 40 units/liter for both transaminases), alkaline phosphatase was 332 units/liter (normal to 115), and serum albumin was 2.3 with a total protein of 6.2 g/dl. A sonogram showed hepatosplenomegaly. Neither dilated ducts nor gallstones were detected.

A needle biopsy of the liver performed on 6.18.84 revealed marked distortion of the lobular architecture. Extensive bridging necrosis of cells with development of a micronodular pattern was present. Cell dropout, ballooning, extensive cytoplasmic hyaline material (Mallory bodies), polymorphonuclear infiltration and patchy fatty metamorphosis were present. The findings were those of an active alcoholic hepatitis with extensive irregular collapse of the parenchyma and the development of an early cirrhosis.

The patient did not consume alcohol. No other known causes of this type of liver histology were present. The lesion was attributed to amiodarone toxicity and the drug was stopped on 6.20.84. On 6.25.84, the patient abruptly developed ventricular fibrillation which was unresponsive to vigorous resuscitation efforts.

Autopsy revealed an enlarged liver (2410 grams), spleen (370 grams) and ascites (1000 ml). The liver was fatty (macro- and microvesicular fat) and disclosed a micronodular cirrhosis containing extensive hyaline necrosis.

The heart revealed an anteroseptal myocardial infarction with organizing apical thrombus. The left anterior descending coronary artery was 95-100% occluded 1 to 2 cm from its origin. The circumflex and right coronary arteries were patent.

Focal pulmonary interstitial fibrosis was present.

Major hepatic features of this case

1. Severe liver disease morphologically resembling alcoholic hepatitis and progressing to micronodular cirrhosis.

2. Slight abnormalities in liver laboratory tests until terminal admission. Changes in liver tests underestimate extent of histologic changes.

A number of other cases of amiodarone-induced liver disease were reported at about the same time as this Dallas case was detected. Features of all of these cases are summarized in Table 1.

TABLE 1

CASES OF AMIODARONE-INDUCED LIVER DISEASE

Case #	Age	Sex	Length of Rx	Hepatomegaly	Bilirubin mg/dl	AST	ALT	AP	Albumin	Liver Biopsy (B) or Autopsy (A)	Mallory Bodies	Multilamellar Bodies
						I.U./liter or X upper limit normal						
Poucell et al (1)												
1	64	F	2 years	+ (19 cm)	0.7	-	1.4X	2.1X	3.8	(B) Micronodular cirrhosis	+	+
2	62	M	2 years	+ (16 cm)	0.5	-	1.3X	1.1X	3.7	(B) Micronodular cirrhosis	-	+
3	61	F	11 months	+		-	3.4X	0.4X				
			14 months	+	1.2	-	11.3X	0.9X	3.6	(B) Hepatocellular alterations with fat, inflammation, some fibrosis	+	+
Lim et al (2)												
4	68	M	21 months	+	1.6	142 IU	-	854 IU		(B) Periportal and central fibrosis; moderate infiltrate, lymph and plasma cells	+	
			died 5 mos later after stopping drug	-	-	30 IU	-	450 IU		(A) Micronodular cirrhosis		
Simon et al (3)												
5	30	M	5 months	-	normal	4.3X	-	normal				
			10 mos later after stopping drug	-	normal	4X	5X	normal		(B) Fibrosis, fat, ballooning, scattered necrosis, focal inflammation, mainly polys	+	+
Present case												
6	76	F	10 months		0.3	182 IU	112 IU	1.1X	3.5			
			14 months	+	3.8	147 IU	68 IU	2.9X	2.3	(A+B) Micronodular cirrhosis	+	+

S Poucell et al. Amiodarone-associated phospholipidosis and fibrosis of the liver. Light, immunohistochemical, and electron microscopic studies. Gastroenterology 86:926-936, 1984.

PK Lim et al. Neuropathy and fatal hepatitis in a patient receiving amiodarone. Br Med J 288:1638-1639, 1984.

JB Simon et al. Admiodarone hepatotoxicity stimulating alcoholic liver disease. New Engl J Med 311:167-172, 1984.

Amiodarone is a benzofuran derivative introduced initially almost twenty years ago as an antianginal agent. Subsequently, it was found to have potent antiarrhythmic effects. Currently it is used largely in the management of patients with ventricular arrhythmias difficult to control with other conventional agents.

Side effects are reported commonly during therapy. Data from five recent reports are summarized in Table 2.

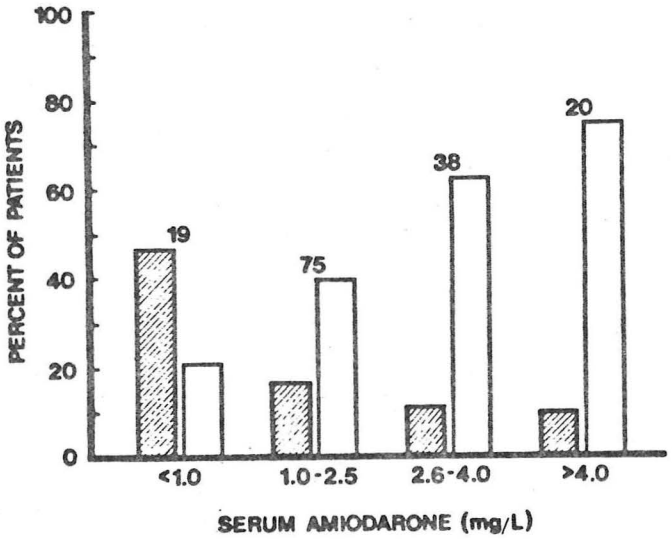
TABLE 2

SIDE EFFECTS DURING AMIODARONE THERAPY

	Heger et al 1981 n=45	Peter et al 1983 n=181	Rakita et al 1983 n=143	McGovern et al 1983 n=80	Rotmensch et al 1984 n=127
Ophthalmologic					
Corneal microdeposits	23/29 (79%)	>75%	all	all	almost all
Photophobia, halo vision, others		12	2	2	18
Abnormal hepatic enzymes	17/31 (55%)	74 (40%)	15/100 (15%)	14/35 (40%)	29 (23%)
Pulmonary infiltrates					
Fibrosing alveolitis	3	8	1	4	4
Neurologic					
Tremor		29	30%	8	4
Peripheral neuropathy	2	1	0		1
Skin					
Photosensitivity		9	57%	7	10
Bluish skin discoloration	3	6	2	3	2
Miscellaneous					
Anorexia, nausea	12	2	31% early	25	9
Weakness, fatigue	7			14	15
Hyperthyroidism	1		2		
Hypothyroidism		8	2	1	5

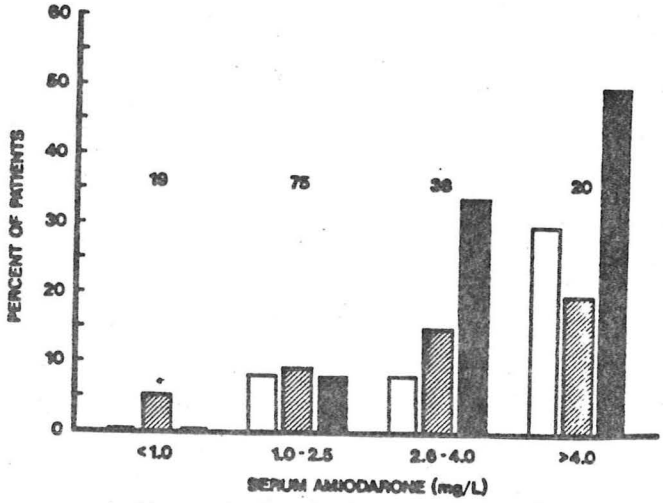
Corneal microdeposits are detected in almost all patients treated for over a few months. These rarely result in visual disturbances, however. Abnormal results of liver tests, usually a 1 to 2 fold increase in SGOT (AST) and SGPT (ALT), are the next most common disturbance. Symptomatic liver disease is uncommon. Liver test abnormalities may improve despite continuation of therapy, and frequently become less abnormal with reduction in dosage. Liver biopsies are rarely obtained. Findings described above in Table 1 in the six patients with major hepatic disease despite modest liver test abnormalities in most of them will undoubtedly lead to additional tissue sampling in the future.

The frequency of adverse effects, particularly those involving liver and nerves, appears to be related to blood levels of the drug.



Incidence of arrhythmia recurrence (*hatched bars*) and adverse effects (*plain bars*) in relation to increasing apparent steady-state serum concentrations of amiodarone. The ordered chi-squared test was done both with and without the repeated measurements and yielded similar results. The overall risk of developing adverse effects increased with increasing concentrations ($p < 0.0001$), whereas patients with serum amiodarone concentrations below 1.0 mg/L were at higher risk of recurrence ($p < 0.005$). Numbers above columns indicate the total number of serum amiodarone determinations in each range obtained from 127 patients, 25 of whom received two maintenance doses for at least 2 months.

From Rotmensch et al, Annals of Internal Medicine 101:462-469, 1984



Incidence of selected adverse effects in relation to increasing serum amiodarone concentrations in 152 observations in 127 patients. With increasing concentrations, risk for hepatic (*solid bars*) ($p < 0.0001$) and neuromuscular (*plain bars*) ($p < 0.01$) effects increased, and a trend ($p = 0.08$) for ocular side effects (*hatched bars*) was noted. Similar statistical results were obtained after exclusion of repeat measurements in these 127 patients.

From Rotmensch et al, Annals of Internal Medicine 101:462-469, 1984

Lamellated bodies similar to those described in the liver have been reported in the cornea, lungs, nerves and skin.

JJ Heger, EN Prystowski, WM Jackman, GV Naccarelli, KA Warfel, RL Rinkenberger and DP Zipes. Clinical efficacy and electrophysiology during long-term therapy for recurrent ventricular tachycardia or ventricular fibrillation. New Engl J Med 305:539-545, 1981.

B McGovern, H Garan, E Kelly and JN Ruskin. Adverse reactions during treatment with amiodarone hydrochloride. Br Med J 287:175-180, 1983.

L Harris, WJ McKenna, E Rowland and DM Krikler. Side effects and possible contraindications of amiodarone use. Am Heart J 106:916-921, 1983.

T Peter, A Hamer, WJ Mandel and D Weiss. Evaluation of amiodarone therapy in the treatment of drug-resistant cardiac arrhythmias: Long-term follow-up. Am Heart J 106:943-947, 1983.

HH Rotmensch, B Belhassen, BN Swanson, D Shoshani, SR Spielman, AJ Greenspon, AM Greenspan, PH Vlasses and LN Horowitz. Steady-state serum amiodarone concentrations: Relationships with antiarrhythmic efficacy and toxicity. Ann Int Med 101:462-469, 1984.

DV Ingram. Ocular effects in long-term amiodarone therapy. Am Heart J 106:902-904, 1983.

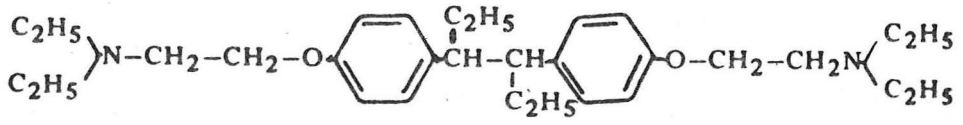
FE Marchlinski, TS Gansler, HL Waxman and ME Josephson. Amiodarone pulmonary toxicity. Ann Int Med 97:839-845, 1982.

L Rakita, SM Sobol, N Mostow and T Vrobel. Amiodarone pulmonary toxicity. Am Heart J 106:906-914, 1983.

JF Lemaire, A Autret, K Biziere, JL Romet-Lemone and F. Gray. Amiodaron Neuropathy: Further arguments for human drug-induced neurolipidosis. Eur Neurol 21:65-68, 1982.

ME Charness, F Morady and MM Scheinman. Frequent neurologic toxicity associated with amiodarone therapy. Neurology 34:6669-6671, 1984.

2. 4,4'-Diethylaminoethoxyhexestrol



Historically the description of liver injury in which a large number of myelin figures filled liver cells emanated from Japan in 1970. The earliest cases were detected hematologically as a type of foam cell syndrome. The liver cases characterized by hepatosplenomegaly and mild liver test abnormalities were soon demonstrated to have increased quantities of phospholipid in the liver, and the entity was termed Phospholipid Fatty Liver. The spectrum of liver histologic findings included cirrhosis with deposition of alcoholic hyaline-like material. Autopsy data also revealed lamellated bodies in the spleen, lymph nodes and bone marrow. It thus became appreciated that foam cell syndrome and phospholipid fatty liver were related to each other.

The earliest lipid analysis of liver tissue revealed disproportionate increases in the acidic phospholipid compounds lysobisphosphatidic acid, now called bis(monoacylglyceryl)phosphate, and phosphatidylinositol. Lipid composition of liver obtained from seven patients is summarized in the following table obtained from the publication of Yamamoto et al (J Biochem 70:775-784, 1971).

Phospholipid fatty liver

Lipid composition of the liver. —, not determined.

Case No.	1				2	3	4		6	7[3]	Normal liver
Date[1]	(2/6/69)	(7/21/69)	(10/2/69)	(4/21/70)			(9/10/70)	(11/10/70)			
Triglyceride	0.83[4]	2.13	1.27	0.24	11.25	1.53	0.12	—	0.22	—	0.47±0.20[5]
Cholesterol ester	0.22	0.35	0.20	0.21	0.28	0.19	0.29	—	0.40	—	0.19±0.08
Free cholesterol	0.68	0.84	0.73	0.48	1.21	1.53	0.93	—	0.72	—	0.17±0.03
Phospholipid	3.50	5.32	4.28	4.36	4.92	5.68	4.53	4.83	4.48	5.24	2.95±0.29
Phosphatidylethanolamine	17.8[2]	17.2	17.2	21.2	17.2	17.4	19.0	17.6	18.4	14.4	28.7 ±1.1
Phosphatidylcholine	46.9	38.3	36.1	34.6	37.8	50.5	40.2	37.6	38.5	48.0	48.5 ±1.0
Phosphatidylinositol	12.6	9.7	9.3	5.0	10.6	14.7	7.3	6.3	10.5	15.8	6.4 ±0.6
Phosphatidylserine	3.3	2.4	2.6	2.6	3.0	3.0	2.9	2.8	2.9	4.2	3.8 ±1.0
Cardiolipin	2.4	2.4	4.0	2.0	2.6	1.8	1.6	1.2	2.0	2.4	4.2 ±1.0
Sphingomyelin	4.2	5.7	5.1	4.2	4.7	4.5	5.8	3.4	5.1	4.6	5.8 ±0.5
Lysophosphatidylcholine	0.5	0.4	0.7	0.6	0.8	0.7	0.7	0.6	0.5	1.6	0.4 ±0.2
Phosphatidic acid	0.4	0.1	0.4	0.3	0.5	0.2	0.5	0.1	0.1	0.3	0.6 ±0.3
Lysobisphosphatidic acid	9.2	21.0	22.8	29.4	20.9	6.9	21.5	29.1	20.8	7.3	0.6 ±0.3
Desmosterol per cent of total sterol	—	4.9	—	—	5.4	trace	trace	—	5.9	3.5	0

[1] Date when biopsy performed. [2] Per cent of total phospholipid. [3] Sample obtained at autopsy. [4] Percent of wet weight of tissue. [5] Average (±standard deviation) of 5 cases.

From Yamamoto et al, J. Biochem. 70:775-784, 1971

Moreover, an increase in lysobisphosphatidic acid was also demonstrated in spleen, muscle, lymph nodes and urinary sediment.

A common feature of the cases not appreciated at first was the discovery that all had been taking a coronary vasodilator drug, 4,4'-diethylaminoethoxyhexestrol, hereafter referred to as DH, for more than 6 months.

T Shikata, T Oda, C Naito, T Kanetaka and H Suzuki. Phospholipid fatty liver: A proposal of a new concept and its electron microscopical study. Acta Path Jap 20:467-486, 1970.

T Oda, T Shikata, C Naito, H Suzuki, T Kanetaka, S Iino, K Miyake, T Sakai, H Onda, K Fujiwara, M Yamanaka, N Shimizu and Y Yoshitoshi. Phospholipid fatty liver: A report of three cases with a new type of fatty liver. Japan J Exp Med 40:127-140, 1970.

A Yamamoto, S Adachi, T Ishibe, Y Shinji, Y Kaki-Uchi, K Seki and T Kitani. Accumulation of acidic phospholipids in a case of hyperlipidemia with hepatosplenomegaly. Lipids 5:566-571, 19790.

A Yamamoto, S Adachi, T Kitani, Y Shinji, K Seki, T Nasu and M Nishikawa. Drug-induced lipidosis in human cases and in animal experiments: Accumulation of an acidic glycerophospholipid. J Biochem 69:613-615, 1971.

A Yamamoto, S Adachi, K Ishikawa, T Yokomura, T Kitani, T Nasu, T Imoto and M Nishikawa. Studies in drug-induced lipidosis: III. Lipid composition of the liver and some other tissues in clinical cases of "Niemann-Pick-like syndrome" induced by 4,4'-diethylaminoethoxyhexestrol. J Biochem 70:775-784, 1971.

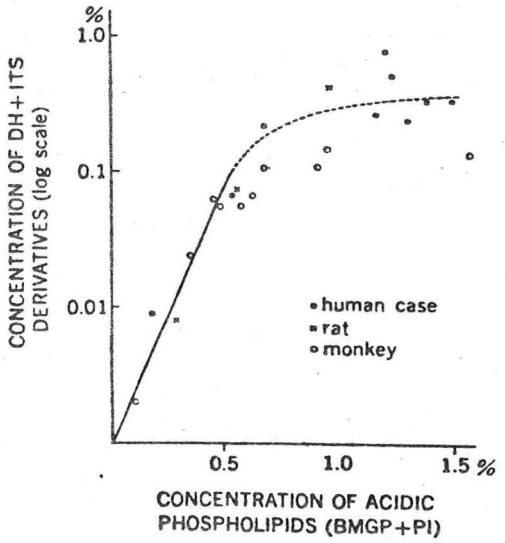
T Shikata, T Kanetaka, Y Endo and K Nagashima. Drug-induced generalized phospholipidosis. Acta Path Jap 22:517-531, 1972.

Subsequent studies have helped to elucidate many of the features of DH-induced phospholipidosis. The bottom line is that accumulation of phospholipids in the liver and presumably in other organs as well is due to drug-induced interference with lysosomal processing of complex lipids in these tissues.

1. DH, when administered to rats and monkeys, leads to accumulation of phospholipids in liver in a pattern similar to that encountered in man.

2. DH itself and some of its metabolites accumulate in liver.

3. A proportionate increase in DH and acidic phospholipids is detected at drug concentrations below 0.1% of the fresh weight of the liver. When drug concentration exceeds 0.1%, a striking further increase in accumulation of acidic phospholipids is found.



Relationship between the concentration of the drug with its derivatives and the concentration of acidic phospholipids (BMGP+PI) in livers of human cases (●), monkeys (○), and rats (×). Values are expressed as percent of the fresh weight of the liver.

From Matsuzawa et al, J. Biochem. 82:1369-1377, 1977

4. A value of 0.1% of drug in liver corresponds to the critical point over which histologic damage in liver becomes extremely severe.

5. DH and its metabolites and the increased phospholipid in liver are present largely in liver lysosomes.

Estimation of the size of intracellular pools of protein and phospholipid in the liver of drug-treated rats

Treatment	Fraction	Protein (mg per fraction)*	Lipid phosphorus (nmol/mg protein)	Total lipid phosphorus per fraction**
Control	Mito	352 ± 59	272 ± 10	96 ± 14
	Micro	269 ± 16	620 ± 22	167 ± 14
	Lyso	26 ± 11	510 ± 54	14 ± 6
DH-treated	Mito	371 ± 44	238 ± 36	87 ± 10
	Micro	268 ± 18	644 ± 39	173 ± 17
	Lyso	76 ± 13[a]	2820 ± 230[b]	215 ± 48[b]

Adapted from Matsuzawa, Y. and Hostetler, K.Y. (1980) Biochim. Biophys. Acta, 620:592-602. Abbreviations: Mito, mitochondria; Micro, microsomes; Lyso, lysosomes. a - $p < .005$; b - $p < .001$ versus control ($n = 3$).

* - mg protein per total fraction assuming 1.0 g total liver protein.

** - mol lipid phosphorus per total fraction.

Intracellular localization of DH in rat liver

Cell fraction	DH, nmol/mg protein	f *
Homogenate	17	1.0
Mitochondria	3	0.2
Lysosomes	189	11.1
Microsomes	7	0.4
Supernatant	4	0.2

Adapted from Matsuzawa, Y. and Hostetler, K.Y. J. Lipid Res., 21:202-214, 1980.

* Enrichment factor (f) relative to the concentration of DH in the cell homogenate.

6. DH at concentrations attained in lysosomes inhibits the action of lysosomal phospholipases responsible for the degradation of phospholipids.

S. Adachi, Y Matsuzawa, T Yokomura, K Ishikawa, S Uhara, A Yamamoto and M Nishikawa. Studies on drug-induced lipodosis. V. Changes in the lipid composition of rat liver and spleen following the administration of 4,4'-diethylaminoethoxyhexestrol. Lipids 7:1-7, 1972.

Y Matsuzawa, T Yokomura, K Ishikawa, S Adachi and A Yamamoto. Studies on drug-induced lipidosis. VI. Identification and determination of the drug and its metabolite in lipidosis induced by 4,4'-diethylaminoethoxyhexestrol. J Biochem 72:615-621, 1972.

A Yamamoto, S Adachi, Y Matsuzawa, T Kitani, A Hiraoka and K Seki. Studies on drug-induced lipidosis: VII. Effects of bis-β-diethylaminoethylether of hexestrol, chloroquine, homochlorocyclizine, prenylamine, and diazacholesterol on the lipid composition of rat lilver and kidney. Lipids 11:616-622, 1976.

Y Matsuzawa, A Yamamoto, S Adachi and M Nishikawa. Studies on drug-induced lipidosis. VIII. Correlation between drug accumulation and acidic phospholipids. J Biochem 82:1368-1377, 1977.

JR Wherrett and S Huterer. Enrichment of bis-(monoacylglyceryl) phosphate in lysosomes from rat liver. J Biol Chem 247:4114-4120, 1972.

Y Matsuzawa, BJHM Poorthuis and KY Hostetler. Mechanism of phosphatidylinositol stimulation of lysosomal bis(monoacylglyceryl)phosphate synthesis. J Biol Chem 253:6650-6653, 1978.

Y Matsuzawa and KY Hostetler. Degradation of bis(monoacylglycero)phosphate by an acid phosphodiesterase in rat liver lysosomes. J Biol Chem 254:5997-6001, 1979.

Y Matsuzawa and KY Hostetler. Studies on drug-induced lipidosis: subcellular localization of phospholipid and cholesterol in the liver of rats treated with chloroquine or 4,4'-bis(diethylaminoethoxy)α,β-diethyldiphenylethane. J Lipid Res 21:202-214, 1980.

Y Matsuzawa and KY Hostetler. Inhibition of lysosomal phospholipase A and phospholipase C by chloroquine and 4,4'-bis(diethylaminoethoxy)α,β-diethyldiphenylethane. J Biol Chem 255:5190-5194, 1980.

Drug-induced lysosomal storage disorders are by no means confined to amiodarone and to DH. Largely as a result of electron microscopic studies which recognized membrane-bound lamellated or crystalline bodies in various tissues, it has been appreciated that a variety of drugs can induce accumulation of phospholipids.

The characteristics of lysosomal storage of polar lipids are as follows:

1. Membrane-bound, acid phosphatase-positive, cytoplasmic inclusions with lamellated or crystalline-like ultrastructure are found in various tissues.

2. Increased amounts of polar lipids are present.

3. The inducing drug accumulates together with the lipids.

4. Reversibility ensues after discontinuance of the drug.

Characteristics of lipidosis-inducing drugs:

1. All are amphiphilic bases

2. They contain a hydrophobic region, usually an aromatic ring or a ring system.

3. They contain a hydrophilic region, usually a short side chain which carries a primary, secondary or tertiary amino group

4. They usually need to be administered for long periods of time.

Compounds which are reported to induce lipidosis are as follows:

Amphiphilic cationic compounds inducing lipidosis

(a) Generalized lipidosis as demonstrated in animals

Compound	*Therapeutic action*
Chlorphentermine^{++} (1)	anorectic
Fenfluramine^{+}	anorectic
R-800 (experimental compound)$^{++}$ (2)	anorectic
Imipramine^{++} (3)	antidepressant
Clomipramine^{++}	antidepressant
Iprindole^{++} (4)	antidepressant
1-Chloro-amitriptyline (exp. compound)$^{++}$ (5)	antidepressant
Zimelidine^{++}	antidepressant
Clozapine $^{+}$ (6)	neuroleptic
AC-3579 (exp. compound)$^{+}$ (7)	tranquillizer, antileucemic
Triparanol^{++} (8)	inhibitor of cholesterol synthesis
AY-9944 (exp. compound)$^{++}$ (9)	inhibitor of cholesterol synthesis
20, 25-Diazacholesterol^{++} (10)	inhibitor of cholesterol synthesis
Mepacrine (Quinacrine)$^{++}$ (11)	antimalarial, antirheumatic
4-Cyano-5-chloro-phenyl-amidinourea (exp. compound)$^{+}$ (12)	antimalarial
IA-3 (exp. compound)$^{+}$ (13)	schistosomicidal
RMI-10.393 (exp. compound)[a] (14)	antithrombotic
Chlorcyclizine^{++} (15)	antihistaminic
Tamoxifen^{++} (16)	antiestrogen

[a]Reports on lung only.

(b) Generalized lipidosis as demonstrated in humans and in animals

Compound	*Therapeutic action*
Chloroquine^{++} (17)	antimalarial, antirheumatic
4,4'-DH (4,4'-Diethyl-aminoethoxyhexestrol)$^{++}$ (18)	antianginal
Amiodarone[b] (19)	antianginal
Perhexiline^{++} (20)	antianginal

[b]For humans: reports on cornea only; in rats: pronounced potency to induce generalized lipidosis.

(c) Drugs with low lipidosis-inducing potency in intact animals, but with pronounced potency to induce lipidosis in cultured cells

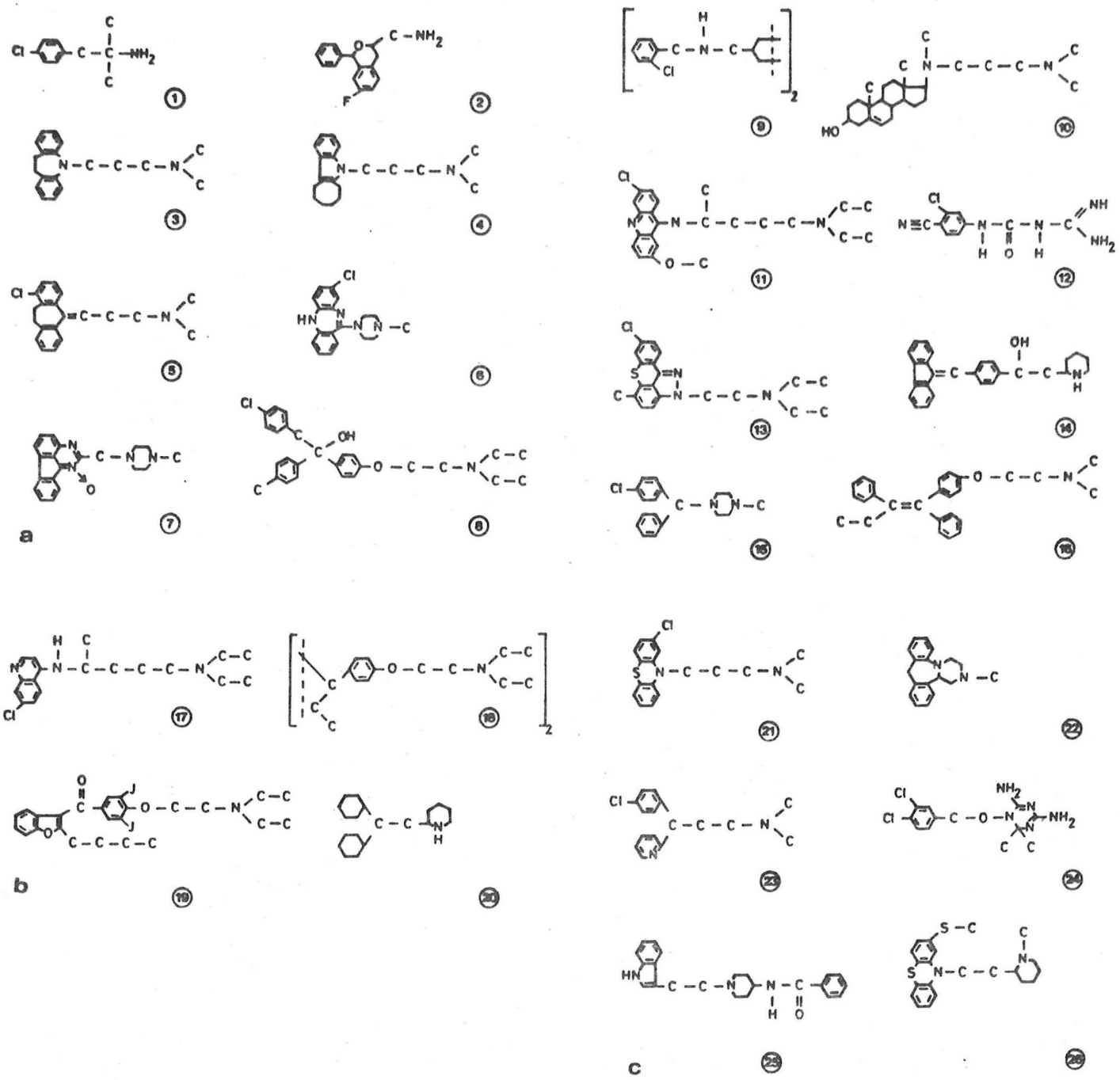
Compound	*Therapeutic action*
Chlorpromazine (21)	neuroleptic
Amitriptyline	antidepressant
Noxiptiline	antidepressant
Mianserine (22)	antidepressant
Maprotiline[c]	antidepressant
Nortriptyline[d]	antidepressant
Chlorpheniramine (23)	antihistaminic
Clociguanil (24)	antimalarial
Indoramine[c] (25)	α-sympatholytic
Thioridazine[c] (26)	neuroleptic
Mesoridazine[d]	neuroleptic
Lysergic acid diethylamide[d]	psychotropic

[c]Reports on liver of intact animals only.
[d]Reports on cultured cells only.
++, pronounced potency to induce generalized lipidosis; +, relatively low potency to induce generalized lipidosis.

Number in parentheses indicates the number of structural formulae listed on the next two pages.

From Luellmann-Rauch, in: Lysosomes in Applied Biology and Therapeutics, 6th Ed., JT Dingle, PJ Jacques, IH Shaw, eds., 1979, North Holland Publishing Co., Amsterdam, N.Y., Oxford, pp. 49-130.

Their formulas are as follows:



Molecular structures of selected compounds included in Table 1. For the sake of clarity the carbon-bound hydrogens are omitted from the formulas. a) Compounds from Table 1a: (1) Chlorphentermine, (2) R-800, *cis*-7-Fluoro-1-phenyl-3-isochromanmethylamine, (3) Imipramine, (4) Iprindole, (5) 1-Chloro-amitriptyline, (6) Clozapine, (7) AC- 3579, 2-N-Methyl-piperazinomethyl-1, 3-diazafluoroanthen-1-oxide, (8) Triparanol, (9) AY-9944, *trans*-1, 4-*bis*-(chlorobenzylaminomethyl)-cyclo-hexane, (10) 20, 25-Diazacholesterol, (11) Mepacrine (Quinacrine), (12) 4-Cyano-5-chlorophenylamidinourea, (13) IA-3, 8-Chloro-5-methyl-2-[2-(diethylamino) ethyl]-2H-[1] benzthiopyranol [4, 3, 2-c, d]-indazol, (14) RMI-10.393, 5-[p-(Fluoren-9-ylidenemethyl)-phenyl]-2-piperidineethanol, (15) Chlorcyclizine, (16) Tamoxifen. b) Compounds from Table 1b: (17) Chloroquine, (18) 4, 4'-Diethylamino-ethoxyhexestrol, (19) Amiodarone, (20) Perhexiline. c) Compounds from Table 1c: (21) Chlorpromazine, (22) Mianserine, (23) Chlorpheniramine, (24) Clociguanil, (25) Indoramine, (26) Thioridazine.

Some of these compounds are very effective in inducing lipidosis in cultured cells but not in intact animals. This is probably accounted for by their metabolism in vivo, i.e. ring hydroxylation. This creates more polar compounds which are less amphiphilic and thus have less lipidosis-inducing effects.

The lysosomes are the site of lipid storage. A hypothetical model to explain the initial events leading to intralysosomal storage is shown below.

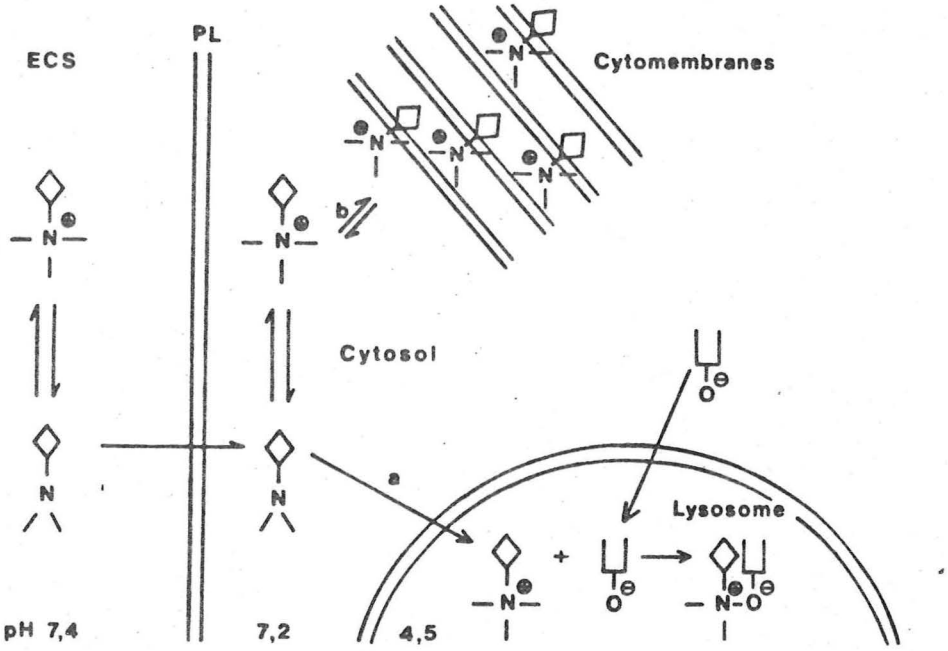


Fig. 11. Hypothetical model proposed as an explanation of the initial events leading to intralysosomal lipid storage (redrawn from Lüllmann et al. 1978). Diagrammatic presentation of cellular uptake, lysosomal accumulation, and membranous adsorption of an amphiphilic drug during the initial phase of exposure. The pK_a of the amine group is considered to be above 7. After passive permeation through the plasmalemma (PL) the drug will be subjected to two competing intracellular processes. 1) The protonized form of the compound will become adsorbed to lipid-water interfaces such as cytomembranes. 2) The non-protonized form will permeate through the lysosomal membrane and will be trapped in the lysosome due to the high proton concentration. Within the lysosome, the cationic amphiphilic compound forms complexes with polar lipids. Since the complexes are removed from the diffusion equilibria, continuous gradients for both the drug and the lipids are sustained leading to a gradual accumulation of drug-lipid complexes. The rate of intralysosomal drug accumulation will depend, among other factors, upon the initial ratio between the two competing intracellular processes (a) and (b). Cationic amphiphilic compounds that undergo relatively little adsorption [process (b) is small compared with process (a)], will display particularly high initial rates of lysosomal accumulation. This may lead to lysosomal swelling due to osmotic events, presumably because the supply of polar lipids as binding partners lags behind, which would otherwise complex with the drug and thus remove it from the osmotic equilibrium.

Symbols: ◇ –N– ◇, amphiphilic drugs; ⊔ phospholipids; ◇ and ⊔, hydrophobic moieties of the respective molecules.

From Luellmann-Rauch, in: Lysosomes in Applied Biology and Therapeutics, 6th Ed., JT Dingle, PJ Jacques, IH Shaw, eds., 1979, North Holland Publishing Co., Amsterdam, N.Y., Oxford, pp. 49-130.

Lipidosis could ensue from decreased catabolism and/or increased synthesis of the polar lipids. Most of the evidence favors the former occurring in cells that normally catabolize significant amounts of polar lipids.

Decreased catabolism of phospholipids:

1. Decreased activity of lysosomal enzymes

   Drug-enzyme interaction

   Alteration in lysosomal pH

2. Decreased digestibility of substrate

   Drug-lipid interaction

R. Luellman-Rauch. Drug-induced lysosomal storage disorders. In: Drug-induced lysosomal storage disorders, Lysosomes in Applied Biology and Therapeutics, 6th Ed., JT Dingle, PJ Jacques and IH Shaw, editors. North Holland Publishing Co., Amsterdam, New York, Oxford, 1979, pp.49-130.

Z Hruban, A Slesers and E Hopkins. Drug-induced and naturally occurring myeloid bodies. Lab Invest 27:62-70, 1972.

KY Hostetler and Y Matsuzawa. Studies on the mechanism of drug-induced lipidosis: Cationic amphiphilic drug inhibition of lysosomal phospholipases A and C. Biochem Pharmacol 30:1121-1126, 1981.

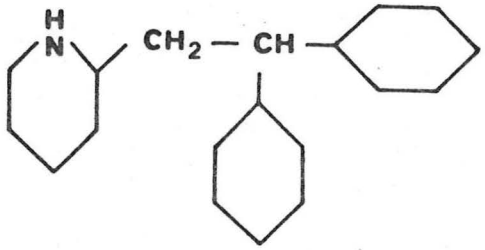
KY Hostetler and DD Richman. Studies on the mechanism of phospholipid storage induced by amantadine and chloroquine in Madin Darby canine kidney cells. Biochem Pharmacol 31:3795-3799, 1982.

C deDuve, T deBarsy, B Poole, A Trouet, P Tulkens and F vanHoof. Lysosomotropic agents. Biochem Pharmacol 23:2495-2531, 1974.

D Drenckhahn and R Luellmann-Rauch. Experimental myopathy induced by amphiphilic cationic compounds including several psychotropic drugs. Neuroscience 4:549-562, 1979.

D Drenckhahn and R Luellmann-Rauch. Drug-induced experimental lipidosis in the nervous system. Neuroscience 4:697-712, 1979.

3. Perhexiline maleate



Perhexiline maleate, a drug widely used in Europe in the treatment of angina pectoris, also produces liver damage similar to that reported for amiodarone and DH.

Features of some of the cases are shown in the accompanying two tables obtained from the report of Pessayre et al, Gastroenterology 76:170-177, 1979.

Other side effects include weight loss, proximal myopathy and peripheral neuropathy.

Liver lesions attributed to perhexiline:

Non-specific hepatitis with or without fat	18
Acute or subacute hepatitis resembling acute alcoholic hepatitis	17
Micronodular cirrhosis with superimposed alcoholic hepatitis	14
Granulomatous hepatitis	2

Ultrastructural lesions include numerous enlarged lysosomes containing myeloid figures. Histochemical stains demonstrate increased phospholipid content of hepatocytes.

Epidemiologic Data and Clinical Features in 7 Patients with Perhexiline Maleate-Induced Cirrhosis

Study	Sex	Age	Perhexiline maleate: Duration of treatment	Perhexiline maleate: Total dose	Other drugs	Jaundice	Hepatic encephalopathy	Ascites	Weight loss	Peripheral neuropathy	Outcome	Survival or follow-up
		yr	mo	g					kg		mo	mo
Pelletier et al.[23] Beaugrand et al.[24]	F	53	15	90	0[a]	+[a]	0	0	24	+	?[a]	?
Case 1	M	66	10	45	Amiodarone; ethyl biscoumacetate	+	+	+	>5	+	Death	3
Case 2	M	53	9	78	Acenocoumarin; pindolol; nitroglycerin	0	0	0	8	0	Inactive cirrhosis	7
Lageron et al.[25]	M	72	15	180	0	0	0	0	10	+	Inactive cirrhosis	15
Bonnet et al.[26]	M	61	33	198	0	+	+	+	10	+	Death	1.5
Pessayre et al. (this study)												
Case 1	M	61	28	168	Acenocoumarin	+	+	+	10	+	Death	6
Case 2	F	67	24	144	Nitroglycerin; pentaerythrityl tetranitrate	+	+	+	13	0	Death	5.5

[a] 0 = absent; + = present; ? = unspecified in the publication.

From D Pessayre et al, Gastroenterology 76:170-177, 1979

Biochemical Data (on Admission) and Liver Lesions in 7 Patients with Perhexiline Maleate-Induced Cirrhosis

Study	Serum bilirubin	Serum glutamic pyruvic trans-aminases	Serum alkaline phos-phatase	Serum albumin	Plasma pro-thrombin	Cirrhosis	Hyaline necrosis	Mallory's bodies	Steatosis	Inflam-mation	Enlarged lysosomes with myeloid figures
	mg/100 ml	*× normal*	*× normal*	*g/100 ml*	*% normal*						
Pelletier et al.[23]	3.0	4	2	2.6	50	+[a]	+	+	0[a]	M[a], P[a]	ND[a]
Beaugrand et al.[24]											
Case 1	1.4	5	1.5	3.0	54	+[b]	+	+	+	M, P	+
Case 2	2.0	7	1	3.0	62	+[b]	+	+	+	M, P	ND
Lageron et al.[25]	1.0	6	1	?[a]	100	+[b]	+	0	+	?	+
Bonnet et al.[26]	2.1	3	1	2.3	?	+	+	+	0	M, P	ND
Pessayre et al. (this study)											
Case 1	2.1	3	1	3.4	46	+	+	+	+	M, P	+
Case 2	14.6	29	1	3.1	53	+	+	0	+	M, P	+

[a] + = present; 0 = absent; M = mononuclear cells; P = polymorphonuclear leucocytes; ND = electron microscopy not done; ? = unspecified in the publication.
[b] Cirrhosis initially absent on the first histologic examination was subsequently present on a follow-up histologic examination.

From D Pessayre et al, Gastroenterology 76:170-177, 1979

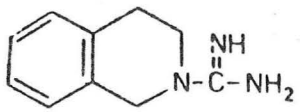
GB Forbes, MO Rake and DJE Taylor. Liver damage due to perhexiline maleate. J Clin Pathol 32:1282-1285.

D Pessayre, M Bichara, G Feldmann, C Degott, Francois Potet and JP Benhamou. Perhexiline maleate-induced cirrhosis. Gastroenterology 76:170-177, 1979.

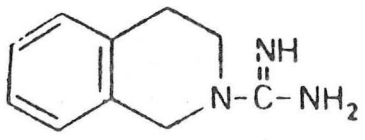
R Poupon, L Rosensztain, P Prudhomme de Saint-Maur, A Lageron, T Gombeau and F Darnis. Perhexiline maleate-associated hepatic injury prevalence and characteristics. Digestion 20:145-150, 1980.

JM Serot, A Floquet, F Penin, G Cuny and G Grignon. Edute ultrastructurale du foie apres traitement par maleate de perhexiline. Sem Hop Paris 53:2199-2200, 1977.

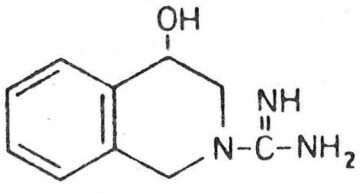
4. Debrisoquine



A forty-fold difference in individual dose requirements for a given decrease in blood pressure was noted for debrisoquine, an antihypertensive drug of the guanethidine type. A positive correlation was observed between drug concentration in plasma and the induced decrease in blood pressure and between the hypotensive effect and unchanged drug excreted in the urine. It was suspected that such differences in drug effect represented an individual variation in the rate of drug metabolism.

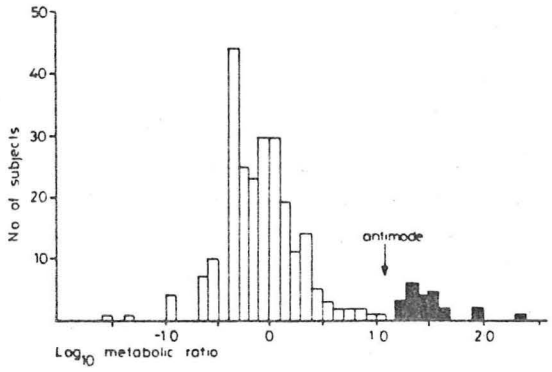


Debrisoquine



4-Hydroxydebrisoquine

Indeed, it was found that the alicyclic hydroxylation of debrisoquine, the process which hastened removal of the drug from the circulation, is expressed as two apparent phenotypes, poor metabolizers (PM), and extensive metabolizers (EM) of debrisoquine.



Frequency distribution of log_{10} metabolic ratio in 258 unrelated white British subjects

From Price-Evans et al, J Med Gen 17:102-105, 1980

8.9% of 258 white unrelated British subjects were poor metabolizers. Family studies revealed that the defective metabolism phenotype is inherited as an autosomal Mendelian recessive character. Extensive metabolism was carried out by persons homozygous or heterozygous for the extensive metabolizer allele. The estimated genotype frequencies in this British population were:

Homozygous	PM	8.9%	(P-P)
Heterozygous	EM	41.9%	(P-E)
Homozygous	EM	49.2%	(E-E)

The approximate dominance of a single EM gene was calculated as about 30%.

Additional studies reveal differences in the incidence of PM in different populations, from a low of 1% in Egyptians to as high as 30% in Orientals.

The deficiency of debrisoquine hydroxylation is associated with decreased metabolism of a number of drugs including perhexiline.

Drugs which undergo polymorphic oxidation of the debrisoquine type:

Sparteine	-	N_1 oxidation
Guanoxan	-	aromatic hydroxylation
Phenacetin	-	oxidative dealkylation
Desmethylimipramine		
Phenytoin	-	p-hydroxylation
Nortriptylene	-	benzylic hydroxylation
Phenformin	-	p-hydroxylation
Perhexiline	-	alicyclic hydroxylation

The deficiency is felt to represent a mute variant of one particular form of cytochrome P450.

Clinical implications of polymorphic drug oxidative reactions:

A percentage of the general population will have increased response and be more prone to side effects of certain drugs when normal or standard doses are administered:

1. Debrisoquine-induced orthostatic hypotension
2. Tricyclic antidepressant-induced cardiotoxicity
3. Sparteine-induced uterine contractions
4. Phenacetin-induced methemoglobinemia
5. Phenformin-induced lactic acidosis

A Mahgoub, JR Idle, LG Dring, R Lancaster and RL Smith. Polymorphic hydroxylation of debrisoquine in man. Lancet ii:584-586, 1977.

DA Price Evans, A Mahgoub, TP Sloan, JR Idle and RL Smith. A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. J Med Gen 17:102-105, 1980.

TP Sloan, A Mahgoub, R Lancaster, JR Idle and RL Smith. Polymorphism of carbon oxidation of drugs and clinical implications. Br Med J 2:655-657, 1978.

M Eichelbaum, N Spannbrucker, B Steincke and HJ Dengler. Defective N-oxidation of sparteine in man: A new pharmacogenetic defect. Eur J Clin Pharmacol 16:183-187, 1979.

M Eichelbaum, L Bertilsson, J Saewe and C Zekorn. Polymorphic oxidation of sparteine and debrisoquine: Related pharmacogenetic entities. Clin Pharmacol Ther 31:184-186, 1982.

T Inaba, A Vinks, SV Otton and W Kalow. Comparative pharmacogenetics of sparteine and debrisoquine. Clin Pharmacol Ther 33:394-399, 1982.

DAP Evans, D Harmer, DY Downham, EJ Whibley, JR Idle, J Ritchie and RL Smith. The genetic control of sparteine and debrisoquine metabolism in man with new methods of analysing bimodal distributions. J Med Gen 20:321-329, 1983.

E Spina, C Birgersson, C von Bahr, O Ericsson, B Mellstroem, E Steiner and Folke Sjoqvist. Phenotypic consistency in hydroxylation of dessmethylimipramine and debrisoquine in healthy subjects and in human liver microsomes. Clin Pharmacol Ther 36:677-682, 1984.

TP Sloan, JR Idle and RL Smith. Influence of D^H/D^L alleles regulating debrisoquine oxidation on phenytoin hydroxylation. Clin Pharmacol Ther 29:493-497, 1980.

L Bertilsson, M Eichelbaum, B Mellstroem, J Saewe, HU Schulz and F Sjoeqvist. Nortriptyline and antipyrine clearance in relation to debrisoquine hydroxylation in man. Life Sciences 27:1673-1677, 1980.

RR Shah, NS Oates, JR Idle and RL Smith. Genetic impairment of phenformin metabolism. Lancet i:1147, 1980.

W Kalow. The metabolism of xenobiotics in different populations. Can J Physiol Pharmacol 60:1-12, 1981.

JW Paxton. Pharmacogenetic polymorphism of drug metabolism. NZ Med J 97L567-569, 1984.

3. Perhexiline maleate

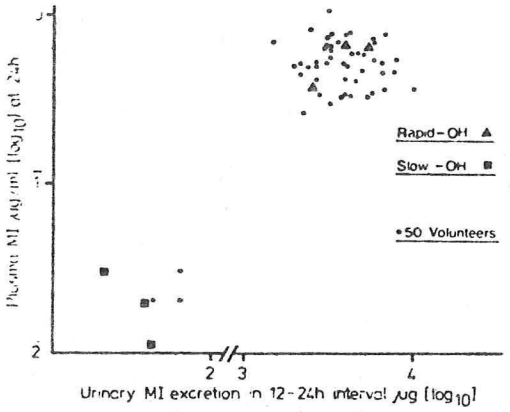
Pertinent to perhexiline-induced phospholipidosis

1. Impaired oxidation of debrisoquine has been demonstrated in patients with perhexiline neuropathy.

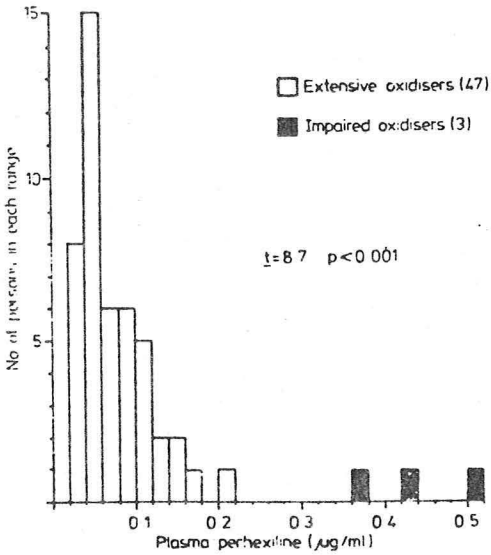
2. Patients with perhexiline neuropathy were demonstrated to have higher plasma levels of perhexiline, slower hepatic metabolism and a longer plasma half-life. Of interest, in this study (Europ. J. Clin. Pharmacol. 14:195-201, 1978), eleven patients, in addition to neuropathy, had signs of hepatic involvement. It was unclear whether hepatic injury accounted for improved perhexiline metabolism.

3. Impaired oxidation of debrisoquine has now been demonstrated in patients with perhexiline liver injury. The impaired oxidation could not be explained by hepatic disease per se.

4. Finally, hydroxylation of perhexiline maleate in man has been shown to be polymorphic and of the debrisoquine type.



The 24-hour plasma concentration of perhexiline MI metabolite concentration and the 12 to 24-hour urinary MI metabolite excretion in 50 random white British subjects of unknown phenotype. Also shown are the results in known extensive (▲) and poor (■) metabolisers of debrisoquine and sparteine.



Plasma 24-hour perhexiline concentrations in 50 random white British subjects.

From Cooper et al, J Med Gen 21:27-33, 1984

RR Shah, NS Oates, JR Idle, RL Smith and JD Flockhart. Impaired oxidation of debrisoquine in patients with perhexiline neuropathy. Brit Med J 284:295-299, 1982.

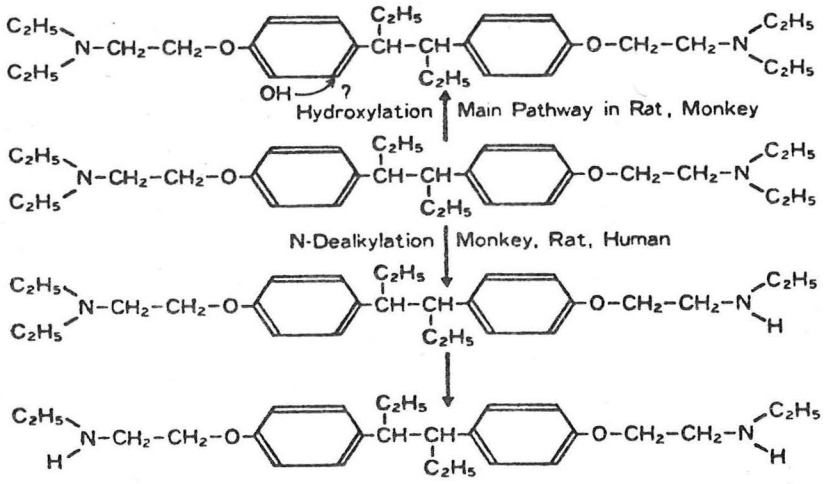
E Singlas, MA Goujet and P Simon. Europ J Clin Pharmacol 14:195-201, 1978.

MY Morgan, R Reshef, RR Shah, NS Oates, RL Smith and S Sherlock. Impaired oxidation of debrisoquine in patients with perhexiline liver injury. Gut 25:1057-1064, 1984.

RG Cooper, CAP Evans and EJ Whibley. Polymorphic hydroxylation of perhexiline maleate in man. J Med Gen 21:27-33, 1984.

It seems reasonably clear that genetically-determined poor metabolism (hydroxylation) of perhexiline leads to accumulation of unmetabolized drug, thereby contributing to the development of toxic side effects.

DH undergoes N-desethylation reactions as well as hydroxylation reactions.

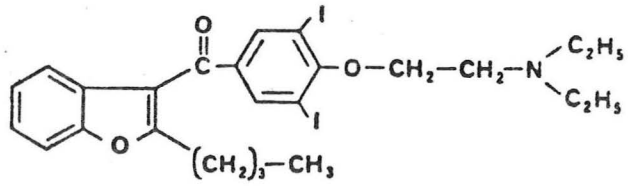


Metabolic pathways of 4,4'-bis(β-diethylaminoethoxy)α,β-diethyldiphenylethane in livers of humans, monkeys, and rats.

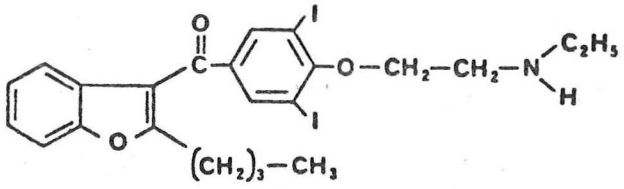
From Matsuzawa et al, J. Biochem. 82:1369-1377, 1977

It is more difficult to induce phospholipidosis with DH in the rat, as compared to the monkey. This may be related to more rapid metabolism, particularly hydroxylation in the rat. Hydroxylated DH metabolites have not been identified in the livers of patients with DH-related hepatotoxicity. No published data exist about DH oxidation in man, nor whether it is polymorphic and of the debrisoquine type. It is of interest that the incidence of polymorphic impaired oxidation of debrisoquine was fairly high in Orientals (mainly Chinese) in one study. DA phospholipidosis is largely an Oriental (Japanese) disease. Investigation of DH metabolism in Japanese and its relationship to DH-induced toxicity would seem to be a worthwhile effort.

Less can be said in this light about amiodarone. The metabolism of this compound is poorly understood. The compound clearly undergoes N-desethylation. Whether it undergoes quantitatively significant oxidation reactions and whether impaired oxidation contributes to its toxicity is unknown. The drug is metabolized very slowly. Whether this is different in those who develop hepato- or neurotoxicity is unclear. These types of toxicity are more common in those with high high blood levels of amiodarone.



Amiodarone



Desethyl amiodarone

DW Holt, GT Tucker, PR Jackson and GCA Storey. Amiodarone pharmacokinetics. Am Heart J 106:840-846, 1983.

HH Rotmensch, B Belhassen, BN Swanson, D Shoshani, SR Spielman, AJ Greenspon, AM Greenspan, PH Vlassses and LN Horowitz. Steady-state serum amiodarone concentrations: Relationships with antiarrhythmic efficacy and toxicity. Ann Int Med 101:462-469, 1984.

The mechanism by which amiodarone, DH and perhexiline produce hepatic lesions resembling those of acute alcoholic hepatitis is unclear. The relationship between phospholipid accumulation, cell necrosis and Mallory body formation is uncertain. They may be causally related or coexistent phenomena.

As of yet there are no data available on whether there is a relationship between those alcohol abusers who develop alcoholic liver disease or the alcoholic hepatitis variant, and to their debrisoquine oxidation status.