MINIREVIEW

Amiodarone Pulmonary Toxicity: Morphologic and Biochemical Features

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Amiodarone was discovered in the early 1960s and originally developed as an antianginal agent (1). Subsequently in 1969, Charlier *et al.* (2) reported that amiodarone exerted antiarrhythmic action in the dog. The first large scale clinical trial was conducted by Rosenbaum *et al.* (3) who noted that amiodarone was effective in the treatment of intractable cardiac arrhythmias. Amiodarone has been approved by the Food and Drug Administration as a cardiac antiarrhythmic agent for the treatment of supraventricular and ventricular arrhythmias. The pharmacology, pharmacokinetics, and interactions with thyroid hormones as well as drug interactions have been the focus of several reviews (4–6) and will not be covered in this article. Although amiodarone is an effective antiarrhythmic agent, drug therapy is unfortunately associated with a high incidence of side effects (7, 8). The most serious, life-threatening adverse effect attributed to amiodarone therapy is pulmonary toxicity characterized by interstitial pneumonitis and fibrosis (8–11). It is the purpose of this review to examine the morphologic and biochemical features of this drug-induced pulmonary toxicity.

Drug Structures

Amiodarone is an iodinated benzofuran derivative that is cationic and amphiphilic in nature. The structure of amiodarone and three of its metabolites, desethylamiodarone, monodeiodinated desethylamiodarone, and bis-desethylamiodarone, are presented in Figure 1.

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Morphologic Features

The pulmonary response to amiodarone treatment is characterized initially by the development of phospholipidosis. Morphologically, this appears as lysosomally derived lamellar bodies in many of the cells of the lungs, including alveolar macrophages (12–15), endothelial and interstitial cells (16–18), bronchiolar epithelial cells (19), and type II cells (12–17). Of these cell types, the alveolar macrophage is particularly susceptible to the development of phospholipidosis. It should be noted that these "foamy" macrophages are present in virtually all patients receiving amiodarone whether toxicity is displayed or not (13, 20, 21). The presence of foamy macrophages and/or lamellar bodies, thus, may not necessarily constitute an index of toxicity.

Reasor *et al.* (14) described the time-dependent response of rat alveolar macrophages to amiodarone treatment. One day after a single dose of amiodarone, a few lamellar structures were typically observed within some of the cells. After 3 days of treatment, lamellar bodies, along with large electron-dense structures were prominent in many cells. Following 1 week of treatment, virtually all of the cells examined contained numerous lamellar bodies, which were associated with areas of amorphous material probably representing a transformation or degeneration of the structures. A representative electron micrograph of an alveolar macrophage from a rat treated with amiodarone is shown in Figure 2. In addition, *in vitro* incubation of rat alveolar macrophages with amiodarone resulted in the development of lamellar bodies which were similar in appearance to those induced by *in vivo* administration of this drug (22).

Biochemical Features

The amiodarone-induced accumulation of foamy macrophages is associated with impaired phospholipid catabolism. Treatment of rats with amiodarone signif-

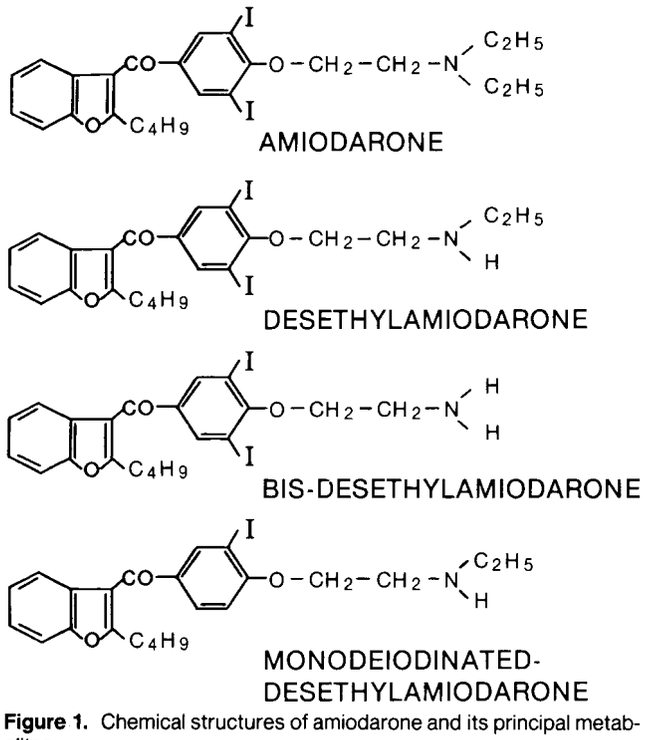

Figure 1. Chemical structures of amiodarone and its principal metabolites.

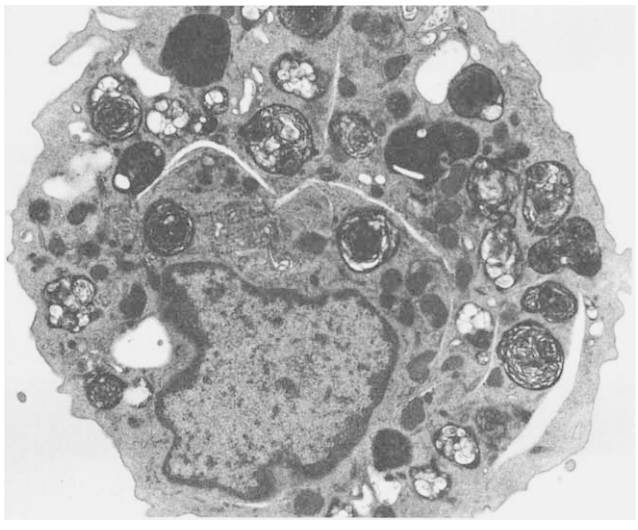

Figure 2. Electron micrograph of an alveolar macrophage from a rat treated with amiodarone for 3 days (150 mg/kg by mouth). Numerous lamellar inclusion bodies and large electron-dense structures are present (original magnification ×17,040). Reproduced with permission of the publisher from (14).

icantly increased the concentration of total phospholipid in whole lung and alveolar macrophages (14, 23–25). The elevation in total phospholipid content was accompanied by a rise in phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, sphingomyelin, and phosphatidylcholine (14, 23, 24). The amiodarone-induced increase in phosphatidylcholine was proportionally greater than all other individual class phospholipids. Similarly, Martin and Standing (13) demonstrated a significant increase in total and individual class phospholipids in bronchoalveolar lavage fluid cells of human subjects with amiodarone pulmonary toxicity. In contrast, no significant increases were observed in the phospholipid composition in the cell-free bronchoalveolar fluid of these same patients.

Within 2 weeks of withdrawal of animals from amiodarone treatment, the phospholipid levels returned to control values, indicating that the phospholipidosis was reversible (14, 23, 26). In contrast, in humans, lamellar inclusion bodies, indicative of phospholipidosis, were present in alveolar macrophages several months after discontinuation of amiodarone treatment (20, 27). At present the basis for the observed interspecies differences in response to amiodarone remains to be determined.

The mechanism by which cationic amphiphilic drugs such as amiodarone induce phospholipidosis is through their ability to inhibit the activity of lysosomal phospholipases (28). It has been shown that amiodarone inhibits phospholipase A_1 of rat lung and alveolar macrophages (29, 30) and inhibits lysosomal phospholipases A_1 and A_2 from rabbit lung (24). The IC_{50} for the amiodarone-induced inhibition of rat lung phospholipase A_1 was 8.7 μM (30) and 11 μM for phospholipase A_2 of rat alveolar macrophages (29). Martin *et al.* (31) demonstrated that the amiodarone-induced inhibition of lysosomal phospholipases A_1 and A_2 was specific in bovine pulmonary artery endothelial cells in that the microsomal phospholipase activities were not altered.

The role of desethylamiodarone, the primary metabolite of amiodarone, in the development of pulmonary phospholipidosis has been the subject of several studies. It is well established that desethylamiodarone accumulates in alveolar macrophages and whole lung tissue of humans (13, 32) and animals (14, 15, 23, 33). Reasor *et al.* (14) demonstrated that lamellar inclusion bodies associated with a significant increase in total phospholipid content were noted in alveolar macrophages collected from rats treated with desethylamiodarone. Likewise, lamellar inclusion bodies were induced in rat alveolar macrophages incubated with desethylamiodarone *in vitro* (22). Additionally, Reasor *et al.* (23) found that with chronic amiodarone treatment, the content of total phospholipid in rat lung correlated more closely with the level of desethylamiodarone in the tissue than with amiodarone. Collectively, these data support the view that desethylamiodarone, as well as amiodarone, contributes to the development of phospholipidosis during amiodarone treatment. Consistent with this theory, Hostetler *et al.* (30) found that desethylamiodarone was slightly more potent than amiodarone in inhibiting rat lung lysosomal phospholipase A_1.

In support of the actions of amiodarone and desethylamiodarone in the induction of phospholipidosis, Kirk *et al.* (34, 35) have reported the distribution of amiodarone and its metabolites in alveolar macrophages following treatment of rats with amiodarone. Because amiodarone and its major metabolites contain iodine, it has been possible to study the intracellular localization of the molecules by monitoring the iodine signal using x-ray microanalysis. While not being able to identify the specific drug species, this technique is able to quantify the amount of iodine associated subcellular structures and is therefore an indication of the total drug level present. Following a single administration of amiodarone, iodine levels were highest in amorphous bodies and dense granules, structures representative of the lysosomal compartment. Lower levels were observed in the cytosol and nucleus of the cell (34) (Table I). The iodine levels in the amorphous bodies rose with increasing treatment time (35). Because of the preferential accumulation of the drugs in the lysosomal compartment where phospholipids accumulate, the results of these studies are consistent with the view that they inhibit phospholipid catabolism. An interesting observation was that by 9 weeks of treatment with amiodarone, the iodine levels in the nucleus approached those found in the amorphous bodies. The significance of the nuclear accumulation of drugs is unclear at present.

Role of Amiodarone Metabolites in Toxicity

Desethylamiodarone and the minor metabolites, monodeiodinated desethylamiodarone, and bis-desethylamiodarone, and at least two unidentified polar metabolites have been detected in blood and tissues of humans (13, 32) and animals (15, 23, 36) treated with amiodarone. It has been mentioned earlier that desethylamiodarone is capable of inducing a phospholipidosis in cells. A number of studies also indicates that desethylamiodarone is more toxic than amiodarone. This has been demonstrated in cell cultures of thyrocytes (37), fibroblasts (37), hepatocytes (38), and alveolar macrophages (22). Ogle and Reasor (22) also showed that when alveolar macrophages were incubated with amiodarone and desethylamiodarone in combination, the cytotoxicity as measured by release of lactate dehydrogenase was greater than additive. Daniels *et al.* (39) reported that desethylamiodarone was more potent than amiodarone in inducing pulmonary fibrosis when administered by intratracheal instillation to hamsters. The results of both *in vitro* and *in vivo* studies are consistent with the view that desethylamiodarone may play a significant, if not the major role in toxicity associated with amiodarone treatment.

Table I. Distribution of Iodine in Alveolar Macrophages of Amiodarone-Treated Rats[a]

	Iodine (mmol/kg dry wt)			
	Control	1 Day[b]	1 Week[c]	9 Weeks[c]
Compartment				
Nucleus	0[d]	10 ± 2	6 ± 3	142 ± 10
Cytosol	1 ± 2	11 ± 2	—[e]	—
Dense granules	0	46 ± 5	—	—
Amorphous bodies	1 ± 2	59 ± 6	116 ± 5	168 ± 11

[a] Values represent mean ± SE; data are from Kirk et al. (34, 35).
[b] Rats were treated with amiodarone (400 mg/kg, by mouth).
[c] Rats were treated with amiodarone (150 mg/kg, by mouth).
[d] When the average values are near zero, small negative and positive values are obtained because of the curve-fitting procedure. Small negative values are presented as zero in the table.
[e] Because of the pronounced phospholipidosis, areas of cytosol were difficult to find, therefore, measurements were not taken. Dense granules were not present.

Mechanisms of Pulmonary Toxicity

While amiodarone administration consistently induces a phospholipidosis in animals and humans, there is no clear indication that this action is related to the development of pulmonary toxicity. Consequently, other mechanisms of toxicity have been proposed. There is evidence of the involvement of immunologic mechanisms in amiodarone-induced pulmonary toxicity. Accordingly, a hypersensitivity mechanism has been proposed to explain these observations as shown in Table II. In contrast, others have found no evidence of immunologic involvement (20, 21, 46, 47).

As previously described, both amiodarone and desethylamiodarone are cytotoxic to a number of cell types *in vitro* (72). As a result of their study with pulmonary artery endothelial cells, Martin and Howard (48) suggested that direct toxicity of amiodarone may contribute to the drug-induced lung damage as evidenced by the induction of cytoplasmic inclusion bodies and the chromium cytotoxicity assay. In agreement Ruben *et al.* (49) demonstrated that incubation of rabbit aorta muscle cells with amiodarone resulted in the induction of dense granules and an elevation in total cellular phospholipid. It has been suggested that lung dysfunction is associated with membrane destabilization. With

Table II. Summary of Amiodarone-Induced Immunologic Responses in Lung

Response	Reference
Deposit of third fragment of complement	(40, 41)
Bronchoalveolar fluid lymphocytosis	(41, 42)
Bronchoalveolar fluid inverted ratio of helper to suppressor T lymphocytes	(41–43)
Positive skin degranulation test	(43)
Basophil degranulation test	(43)
Positive lymphoblastic transformation	(43)
Leukocyte inhibitory factor secretion	(43, 44)
Antibodies reacting with lung tissue of a patient with amiodarone-induced pulmonary fibrosis	(45)

the use of fluorescence polarization, Chatelain *et al.* (50) found that amiodarone was deeply buried into the lipid matrix of membrane vesicles and that only the ionized form of the drug was capable of destabilization of the lipid matrix organization (51). In contrast, Jendrasiak *et al.* (71) found that amiodarone was present in the polar headgroup regions of the liposomal bilayer. The recent findings that amiodarone accumulates into two distinct pools with pool 1 being small and possessing a short half-life, while pool 2 has a longer half-life might account for these differences (73).

Whether amiodarone is buried deep or near the surface (in pool 1 or 2), the consequences associated with perturbations in the cell membranes would be reflected as a change in capillary permeability and manifested by the appearance of pulmonary edema (12, 21, 52). Biochemically, Na^+,K^+-ATPase is a membrane enzyme involved in ionic and amino acid transport systems and thus may be considered as a marker of membrane function (53). Treatment of rats with amiodarone was found to significantly reduce pulmonary Na^+,K^+-ATPase activity (23). In support of the view that amiodarone-induced toxicity is related to a change in membrane transport, Powis *et al.* (54) recently demonstrated that amiodarone increased intracellular calcium levels in human pulmonary endothelial cells. The increased levels of intracellular calcium were suggested to be related to an elevated influx of calcium across the cell plasma membrane. This resultant rise in calcium was thus responsible for cell injury. Similarly, amiodarone decreased the activity of Na^+,K^+-ATPase in rat brain synaptosomes (55, 56) and guinea pig ventricular muscle (57, 58). It is of interest that amiodarone treatment increased the activity of β-*N*-acetylglucosaminidase, a hydrolytic enzyme, in rat lung (23).

Although the clinical relevance of this finding remains to be resolved, excess hydrolytic activity in the lungs may contribute to pulmonary toxicity. Kennedy *et al.* (59) have speculated that amiodarone causes lung damage via an oxidant mechanism. Using an isolated perfused rabbit lung, they observed that amiodarone-induced lung edema was prevented by pretreatment of rabbits with butylated hydroxytoluene, or vitamin E, or addition of *N*-acetylcysteine to the lung perfusate.

Although a number of mechanisms have been implicated in the origin of pulmonary toxicity associated with amiodarone treatment, the relative contributions of each are not clear. It is likely that the development of lung damage is multifactorial, dependent, in part, on individual characteristics of the patient.

Animal Models of Pulmonary Fibrosis

The development of interstitial fibrosis is the most serious aspect of amiodarone-induced pulmonary toxicity (10, 11). While the use of animals has provided important information on the actions of amiodarone and desethylamiodarone, there is little evidence to suggest that systemic amiodarone administration induces pulmonary fibrosis in animals (24, 25, 60). However, delivery of amiodarone or desethylamiodarone to hamster lungs by intratracheal instillation is effective in inducing fibrosis (61–63). Cantor *et al.* (61, 62) demonstrated that intratracheal amiodarone administration to hamsters results in morphologic evidence of fibrosis, a marked deposition of collagen as manifested by trichrome staining and increased elastin synthesis. In agreement with these findings, Daniels *et al.* (63) observed that intratracheal amiodarone or desethylamiodarone administration to hamsters produced pulmonary fibrosis as evidenced by morphologic alterations, trichrome staining, and an increase in lung hydroxyproline content. Additional efforts are required in developing animal models for the study of the actions of amiodarone and desethylamiodarone on the lung.

Amiodarone-Induced Effects on Other Tissues

Although the primary focus of this review is the relationship between amiodarone and pulmonary toxicity, one cannot neglect the fact that amiodarone accumulates in a variety of tissues. High concentrations of amiodarone and desethylamiodarone have been found in lung, liver, spleen, and kidney, lesser amounts in heart, and only trace amounts in brain of rabbits (64) and humans (32). Recently, Kacew and Reasor (65) reported that in rats the tissue levels of amiodarone and desethylamiodarone were highest in lung followed by kidney, liver, heart, and brain in descending order. Of these tissues, total phospholipid was increased and Na^+,K^+-ATPase activity was decreased only in the lungs. Clearly, there is a consensus that the presence of amiodarone in lung is related to toxicity.

However, the relationship between organ toxicity and drug presence is controversial in the case of liver and heart (Table III). Although evidence indicates that amiodarone and desethylamiodarone accumulate in significant amounts in various tissues, including lung, pulmonary tissue appears to consistently be a primary target tissue for biochemical alterations.

Conclusions

Since the discovery of amiodarone over 30 years ago, the mechanisms underlying serious adverse effects remain to be resolved and still limit drug usefulness. Morphologic and biochemical studies indicate that amiodarone exerts an adverse effect predominantly on pulmonary tissue. There is a growing body of evidence supporting the role of the metabolite, desethylamiodarone, in lung toxicity resulting from amiodarone administration. Additionally, there is evidence to indicate that direct cytotoxicity, cellular transport mechanisms, immunologic processes, and oxidant action may play a role in amiodarone-induced pulmonary toxicity. This

Table III. Summary of Amiodarone Effect on Various Tissues

Tissue	Experimental finding	Reference	
		Presence	No change
Liver	Lamellar inclusion bodies index of phospholipidosis	68	15,38,39,66,67
	Serum transaminase activities (function test)	—	39,67
	Na^+, K^+-ATPase	—	65
	Total phospholipid content	—	69
Heart	Increase in total phospholipid	70	65
	Na^+, K^+-ATPase	—	65

review demonstrates that further study is necessary for understanding the actions of amiodarone and desethylamiodarone on lung tissue.

1. Singh BN. Amiodarone: Historical development and pharmacological profile. Am Heart J **106**:788–797, 1983.
2. Charlier R, Delaunois G, Bauthier J, Deltour G. Recherches dans la série des benzofuranes. XL. Propriètés antiarythmiques de l'amiodarone. Cardiologia **54**:83–90, 1969.
3. Rosenbaum MB, Chiale PA, Halpern MS, Nau GJ, Przbylski J, Levi RJ, Lazzari JO, Elizari MO. Clinical efficacy of amiodarone as an antiarrhythmic agent. Am J Cardiol **38**:934–944, 1976.
4. Nattel S, Talajic M. Recent advances in understanding the pharmacology of amiodarone. Drugs **36**:121–131, 1988.
5. Weir SJ, Ueda CT. Amiodarone pharmacokinetics. III. Influence of thyroid dysfunction on amiodarone absorption and disposition. J Pharmacol Exp Ther **246**:1026–1032, 1988.
6. Lesko LJ. Pharmacokinetic drug interactions with amiodarone. Clin Pharmacokinet **17**:130–140, 1989.
7. McGovern B, Garan H, Kelly E, Ruskin JN. Adverse reactions during treatment with amiodarone hydrochloride. Br Med J **287**:175–180, 1983.
8. Mason JW. Amiodarone. N Engl J Med **316**:455–465, 1987.
9. Akoun GM, Liote G, Milleron BJ, Mayaud CM. Amiodarone lung. In: Hollinger MA, Ed. Current Topics in Pulmonary Pharmacology and Toxicology. New York: Elsevier Science Publishing Co., Vol **2**: pp176–194, 1987.
10. Martin WJ II, Rosenow EC III. Amiodarone pulmonary toxicity. Recognition and pathogenesis (part 1). Chest **93**:1067–1075, 1988.
11. Martin WJ II, Rosenow EC III. Amiodarone pulmonary toxicity (Part 2). Recognition and pathogenesis. Chest **93**:1242–1248, 1988.
12. Costa-Jussa FR, Corrin B, Jacobs JM. Amiodarone lung toxicity: A human and experimental study. J Pathol **143**:73–79, 1984.
13. Martin WJ II, Standing JE. Amiodarone pulmonary toxicity: Biochemical evidence for a cellular phospholipidosis in the bronchoalveolar lavage of human subjects. J Pharmacol Exp Ther **244**:774–779, 1988.
14. Reasor MJ, Ogle CL, Walker ER, Kacew S. Amiodarone-induced phospholipidosis in rat alveolar macrophages. Am Rev Respir Dis **137**:510–518, 1988.
15. Kannan R, Sarma JSM, Guha M, Venkataraman K. Tissue drug accumulation and ultrastructural changes during amiodarone administration in rats. Fundam Appl Toxicol **13**:793–803, 1989.
16. Myers JL, Kennedy JI, Plumb VJ. Amiodarone lung: Pathologic findings in clinically toxic patients. Hum Pathol **18**:349–354, 1987.
17. Marchlinski FE, Gansler TS, Waxman HL, Josephson ME. Amiodarone pulmonary toxicity. Ann Intern Med **97**:839–845, 1982.
18. Dake MD, Madison JM, Montgomery CK, Shellito JE, Hinchcliffe WA, Winkler ML, Bainton DF. Electron microscopic demonstration of lysosomal inclusion bodies in lung, liver, lymph nodes, and blood leukocytes of patients with amiodarone pulmonary toxicity. Am J Med **78**:506–512, 1985.
19. Colgan T, Simon GT, Kay JM, Pugsley SO, Eydt J. Amiodarone pulmonary toxicity. Ultrastruct Pathol **6**:199–207, 1984.
20. Liu FL, Cohen RD, Downar E, Butany JW, Edelson JD, Rebuck AS. Amiodarone pulmonary toxicity: Functional and ultrastructural evaluation. Thorax **41**:100–105, 1986.
21. Kennedy JI, Myers JL, Plumb VJ, Fulmer JD. Amiodarone pulmonary toxicity: Clinical, radiologic, and pathologic correlations. Arch Intern Med **147**:50–55, 1987.
22. Ogle CL, Reasor MJ. Response of alveolar macrophages to amiodarone and desethylamiodarone, in vitro. Toxicology **62**:227–238, 1990.
23. Reasor MJ, Ogle CL, Kacew S. Amiodarone-induced pulmonary toxicity in rats: Biochemical and pharmacological characteristics. Toxicol Appl Pharmacol **97**:124–133, 1989.
24. Heath MF, Costa-Jussa FR, Jacobs JM, Jacobson W. The induction of pulmonary phospholipidosis and the inhibition of lysosomal phospholipases by amiodarone. Br J Exp Pathol **66**:391–397, 1985.
25. Riva E, Marchi S, Pesenti A, Bizzi A, Cini M, Vneroni E, Tavbani E, Boeri R, Bertani T, Latini R. Amiodarone induced phospholipidosis: Biochemical, morphological and functional changes in the lungs of rats chronically treated with amiodarone. Biochem Pharmacol **36**:3209–3214, 1987.
26. Mazue G, Vic P, Gouy D, Remandet B, Lacheretz F, Berthe J, Barchewitz G, Gagnol JP. Recovery from amiodarone-induced lipidosis in laboratory animals: a toxicological study. Fundam Appl Toxicol **4**:992–999, 1984.
27. Israel-Biet D, Venet A, Caubarrere I, Bonan G, Danel C, Chretien J, Hance AJ. Bronchoalveolar lavage in amiodarone pneumonitis: Cellular abnormalities and their relevance to pathogenesis. Chest **91**:214–221, 1987.
28. Hostetler KY. Molecular studies of the induction of cellular phospholipidosis by cationic amphiphilic drugs. Fed Proc **43**:2582–2585, 1984.
29. Hostetler KY, Reasor MJ, Walker ER, Yazaki PJ, Frazee BW. Role of phospholipase A inhibition in amiodarone pulmonary toxicity in rats. Biochim Biophys Acta **875**:400–405, 1986.
30. Hostetler KY, Giordano JR, Jellison EJ. In vitro inhibition of lysosomal phospholipase A_1, of rat lung by amiodarone and desethylamiodarone. Biochim Biophys Acta **959**:316–321, 1988.
31. Martin WJ II, Kachel DL, Vilen T, Natarajan V. Mechanism of

phospholipidosis in amiodarone pulmonary toxicity. J Pharmacol Exp Ther **251**:272–278, 1989.
32. Brien JF, Jimmo S, Brennan FJ, Ford SE, Armstrong PW. Distribution of amiodarone and its metabolite, desethylamiodarone, in human tissues. Can J Physiol Pharmacol **65**:360–364, 1987.
33. Camus P, Mehendale HM. Pulmonary sequestration of amiodarone and desethylamiodarone. J Pharmacol Exp Ther **237**:867–873, 1986.
34. Kirk RG, Reasor MJ, Lee P. Iodine in rat alveolar macrophages following amiodarone treatment: Quantitative x-ray microanalysis. Exp Mol Pathol **49**:339–347, 1988.
35. Kirk RG, Lee P, Reasor MJ. Quantitative x-ray microanalysis of alveolar macrophages after long-term treatment with amiodarone. Exp Mol Pathol **53**:122–131, 1990.
36. Bandyopadhyay S, Somani P. A comparison of plasma, white blood cell, red blood cell, and tissue distribution of amiodarone and desethylamiodarone in anesthetized dogs. J Cardiovasc Pharmacol **10**:379–388, 1987.
37. Beddows SA, Page SR, Taylor AH, McNerney R, Withley G St. J, Johnstone AP, Nussey SS. Cytotoxic effects of amiodarone and desethylamiodarone on human thyrocytes. Biochem Pharmacol **38**:4397–4403, 1989.
38. Gross SA, Bandyopadhyay S, Klaunig JE, Somani P. Amiodarone and desethylamiodarone toxicity in isolated hepatocytes in culture. Proc Soc Exp Biol Med **190**:163–169, 1989.
39. Daniels JM, Leeder RG, Brien JF, Massey TE. Repeated amiodarone exposure in the rat: Toxicity and effects on hepatic and extrahepatic monooxygenase activities. Can J Physiol Pharmacol **68**:1261–1268, 1990.
40. Suarez LD, Poderoso JJ, Elsner B, Bunster AM, Esteva H, Bellotti M. Subacute pneumopathy during amiodarone therapy. Chest **83**:566–568, 1983.
41. Manicardi V, Bernini G, Bossini P, Bertorelli G, Pesci A, Bellodi G. Low-dose amiodarone-induced pneumonitis: Evidence of an immunologic pathogenetic mechanism. Am J Med **86**:134–135, 1989.
42. Venet A, Caubarrere I, Bonan G. Five cases of immune-mediated amiodarone pneumonitis. Lancet **1**:962–963, 1984.
43. Akoun GM, Gauthier-Rahman S, Milleron BJ, Perrot JY, Mayaud CM. Amiodarone-induced hypersensitivity pneumonitis: Evidence of an immunological cell-mediated mechanism. Chest **85**:133–135, 1984.
44. Akoun GM, Gauthier-Rahman S, Liote HA, Milleron BJ, Mayaud CM. Leukocyte migration inhibition in amiodarone-associated pneumonitis. Chest **94**:1050–1053, 1988.
45. Fan K, Bell R, Eudy S, Fullenwider J. Amiodarone-associated pulmonary fibrosis: Evidence of an immunologically-mediated mechanism. Chest **92**:625–630, 1987.
46. Adams PC, Gibson GJ, Morley AR, Wright AJ, Corris PA, Reid DS, Campbell RWF. Amiodarone pulmonary toxicity: Clinical and subclinical features. Q J Med **59**:449–471, 1986.
47. Dean PJ, Groshart KD, Porterfield JG, Iansmith DH, Golden EB Jr. Amiodarone-associated pulmonary toxicity. A clinical and pathologic study of eleven cases. Am J Clin Pathol **87**:7–13, 1987.
48. Martin WJ II, Howard DM. Amiodarone-induced lung toxicity. In vitro evidence for the direct toxicity of the drug. Am J Pathol **120**:344–350, 1985.
49. Ruben Z, Anderson SN, Kacew S. Intracellular drug storage: Relationship to alterations in saccharide and phospholipid content in cultured cells. Toxicologist **10**:46, 1990.
50. Chatelain P, Ferreira J, Laurel R, Ruysschaert JM. Amiodarone induced modifications of the phospholipid physical state. A fluorescence polarization study. Biochem Pharmacol **35**:3007–3013, 1986.
51. Ferreira J, Chatelain P, Caspers J, Ruysschaert JM. Ionization state of amiodarone mediates its mode of interaction with lipid bilayers. Biochem Pharmacol **36**:4245–4250, 1987.
52. Darmanata JI, Van Zandwijk N, Duren DR, Van Royen EA, Mooi WJ, Plomp TA, Jansen HM, Durrer D. Amiodarone pneumonitis: Three further cases with a review of published reports. Thorax **39**:57–64, 1984.
53. Cross CE, Parsons GH, Gorin AB, Last JA. Pulmonary edema: Emphasis on physiologic and toxicological considerations. In: Witschi H, Nettesheim P, Eds. Mechanisms in Respiratory Toxicology. Boca Raton, FL: CRC Press, pp219–246, 1982.
54. Powis G, Olsen R, Standing JE, Kachel D, Martin WJ II. Amiodarone-mediated increase in intracellular free Ca^{2+} associated with cellular injury to human pulmonary artery endothelial cells. Toxicol Appl Pharmacol **103**:156–164, 1990.
55. Chatelain P, Laurel R, Gillard M. Effect of amiodarone on membrane fluidity and Na^+,K^+-ATPase activity in rat brain synaptic membranes. Biochem Biophys Res Commun **129**:148–154, 1985.
56. Prasada Rao KS, Rao SB, Camus PH, Mehendale HM. Effect of amiodarone on Na^+-K^+ATPase and Mg^{2+}-ATPase activities in rat brain synaptosomes. Cell Biochem Funct **4**:143–151, 1986.
57. Aomine M. Suggestive evidence for inhibitory action of amiodarone on Na^+, K^+-pump activity in guinea pig heart. Gen Pharmacol **20**:491–496, 1989.
58. Broekhuysen J, Clinet M, Delisee C. Action of amiodarone on guinea pig heart sodium and potassium activated adenosine triphosphatase: Comparison with ouabain. Biochem Pharmacol **21**:2951–2960, 1972.
59. Kennedy TP, Gordon GB, Paky A, McShane A, Adkinson NF, Peters SP, Friday K, Jackman W, Sciuto AM, Gurtner GH. Amiodarone causes acute oxidant lung injury in ventilated and perfused rabbit lungs. J Cardiovasc Pharmacol **12**:23–36, 1988.
60. Wilson BD, Jaworski AJ, Donner ME, Lippmann ML. Amiodarone-induced pulmonary toxicity in the rat. Lung **167**:301–311, 1989.
61. Cantor JO, Osman M, Cerreta JM, Suarez R, Mandl I, Turino GM. Amiodarone-induced pulmonary fibrosis in hamsters. Exp Lung Res **6**:1–10, 1984.
62. Cantor JO, Keller S, Mandl I, Turino GM. Increased synthesis of elastin in amiodarone-induced pulmonary fibrosis. J Lab Clin Med **109**:480–485, 1987.
63. Daniels JM, Brien JF, Massey TE. Pulmonary fibrosis induced in the hamster by amiodarone and desethylamiodarone. Toxicol Appl Pharmacol **100**:350–359, 1989.
64. Kannan R, Miller S, Singh BN. Tissue uptake and metabolism of amiodarone after chronic administration in rabbits. Drug Metab Dispos **13**:646–650, 1985.
65. Kacew S, Reasor MJ. Amiodarone-induced pulmonary toxicity in rats: A tissue-specific manifestation. Toxicologist **10**:46, 1990.
66. Young RA, Mehendale HM. Effects of short-term and long-term administration of amiodarone on hepatobiliary function in male rats. J Appl Toxicol **9**:407–412, 1989.
67. Lambert C, Lamontagne D, Hottlet H, Du Souich P. Amiodarone-digoxin interaction in rats. A reduction in hepatic uptake. Drug Metab Dispos **17**:704–708, 1989.
68. Lewis JH, Mullick F, Ishak KG, Ranard RC, Ragsdale B, Perse RM, Rusnock EJ, Wolke A, Benjamin SB, Seeff LB, Zimmerman HJ. Histopathologic analysis of suspected amiodarone hepatotoxicity. Hum Pathol **21**:59–67, 1990.
69. Pirovino M, Honegger U, Muller O, Zysset T, Kupfer A, Tinel M, Pessayre D. Difference in hepatic drug accumulation and enzyme induction after chronic amiodarone feeding of two rat strains: Role of the hydroxylator phenotype? Br J Pharmacol **99**:35–40, 1990.
70. Shaikh NA, Downar E. Effects of chronic amiodarone treatment

on cat myocardial phospholipid content and on in vitro phospholipid catabolism. Mol Cell Biochem **78**:17–25, 1987.
71. Jendrasiak GL, McIntosh TJ, Ribeiro A, Porter RS. Amiodarone-liposome interaction: A multinuclear NMR and x-ray diffraction study. Biochim Biophys Acta **1024**:19–31, 1990.
72. Wilson BD, Lippmann ML. Pulmonary accumulation of amiodarone and *N*-desethylamiodarone. Relationship to the development of pulmonary toxicity. Am Rev Respir Dis **141**:1553–1558, 1990.
73. Camus P, Coudert B, D'Athis P, Dumas M, Escousse A, Jeannin L. Pharmacokinetics of amiodarone in the isolated rat lung. J Pharmacol Exp Ther **254**:336–343, 1990.