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ACCUMULATION OF AMIODARONE AND DESETHYLAMIODARONE BY RAT ALVEOLAR MACROPHAGES IN CELL CULTURE

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Abstract—Amiodarone is a clinically effective antiarrhythmic drug shown to cause lung damage in humans and animals. While the mechanism of this pulmonary toxicity is unknown, it may be associated with the accumulation of amiodarone and its principal metabolite, desethylamiodarone, by alveolar macrophages. In the present study, characteristics of the uptake of these drugs by rat alveolar macrophages *in vitro* were examined. The alveolar macrophages were collected by pulmonary lavage from male Fischer 344 rats. Amiodarone and desethylamiodarone were incubated separately (2.5 μM) with the cells in culture for 1, 2, 4 and 18 hr. High performance liquid chromatography was used to measure drug uptake. At 1 and 2 hr, the uptake of desethylamiodarone by alveolar macrophages was significantly greater ($P < 0.05$) than that of amiodarone, but over time, the accumulation of amiodarone began to approach that of desethylamiodarone and was not significantly different by 4 hr. To simulate a more physiological situation, plasma levels achieved in the adult male rat after 1 week of amiodarone treatment (150 mg/kg) were used. Amiodarone (1.95 μg/mL) and desethylamiodarone (0.80 μg/mL) were added together into the cell culture. At 1 and 18 hr, the ratio of desethylamiodarone/amiodarone uptake was significantly greater ($P < 0.05$) than in incubation medium containing no cells, indicating an enhanced uptake of desethylamiodarone. Metabolic inhibitors (KCN, 2,4-dinitrophenol, and ouabain) and other cationic, amphiphilic drugs (chlorcyclizine, chlorphentermine, and imipramine) were added individually to the cell cultures containing amiodarone or desethylamiodarone. During 1 hr of incubation, these agents had no effect in blocking the accumulation of amiodarone and desethylamiodarone in the cells. The efflux of amiodarone or desethylamiodarone was measured from cells following incubation for 4 hr with each drug. After this time, the medium was replaced with drug-free medium, and the cells were incubated for another 24 hr. Sixty-three percent of amiodarone was lost as compared to only 31% of desethylamiodarone over the 24-hr period ($P < 0.05$). The results of this study are suggestive of a preferential uptake and retention of desethylamiodarone as compared to amiodarone. The accumulation of the drugs appears not to be due to active transport or associated with any carrier protein involved in the transport of other structurally-related compounds.

Amiodarone, a cationic, amphiphilic drug, is approved for use in the treatment of life-threatening ventricular tachyarrhythmias. It is also effective, but unapproved, in managing less severe ventricular and supraventricular arrhythmias [1]. The limitations for the use of amiodarone are due to its numerous side-effects, the most serious being a dose-dependent pulmonary toxicity [2–4]. With chronic use, patients may develop pulmonary infiltrates characterized as pneumonitis that can progress into life-threatening pulmonary fibrosis. The mechanism by which this lung toxicity occurs is still unclear.

Evidence suggests that there may be an immunological mechanism involved with this pulmonary damage [2, 5, 6]. In contrast, others have found no evidence of immunological involvement [7–10]. The possibility of a non-immunologic component of amiodarone-induced lung injury exists and is based on the presence of lamellar inclusions nearly always found in alveolar lavages and pulmonary biopsy samples [2]. The administration of amiodarone to humans and animals has led to the development of a biochemical and morphological phospholipidosis in many cell types of the lung [4, 11]. These include alveolar macrophages [11, 12], endothelial cells [4], interstitial cells [13], and bronchial epithelial cells [14]. This development of lung injury in humans and animals is associated with a significant accumulation of amiodarone and its principal metabolite, desethylamiodarone, in lung tissue [12, 15, 16]. Recent evidence now shows that the alveolar macrophages play a prominent role in the pulmonary sequestration of both of these drugs and the development of the resulting phospholipidosis [17].

Results of both *in vitro* and *in vivo* studies seem to indicate that desethylamiodarone may play a significant, if not more important, role than its parent compound in the toxicity resulting from amiodarone treatment. Studies using alveolar macrophages have demonstrated that desethylamiodarone is more cytotoxic and induces the formation of lamellar inclusions more rapidly than amiodarone [18]. It also has been reported that desethylamiodarone is more potent than amiodarone in inducing pulmonary fibrosis when administered by intratracheal instillation to hamsters [19].

In this present investigation, we utilized primary cell cultures of rat alveolar macrophages to characterize the ability of these cells to accumulate

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and retain amiodarone and desethylamiodarone. This *in vitro* system has proven to be advantageous in quantifying the uptake of these drugs since metabolism of the compounds does not occur in rat alveolar macrophages [18]. With the use of metabolic inhibitors and other cationic, amphiphilic drugs, we also examined the possible transport mechanisms which may be involved in the uptake of both amiodarone and desethylamiodarone.

MATERIALS AND METHODS

Drugs. Amiodarone, desethylamiodarone, and the HPLC internal standard, L8040, were gifts from Wyeth-Ayerst Research, Princeton, NJ, and Dr. C. Lafille, Sanofi Recherches Centre, Montpellier, France. The following drugs also were provided as gifts: imipramine (Ciba-Geigy Co., Summit, NJ), chlorcyclizine (Burroughs-Wellcome Co., Research Triangle Park, NC) and chlorphentermine (Warner-Chilcott Co., Morris Plains, NJ). KCN (Fischer Scientific Co., Fair Lawn, NJ) and 2,4-dinitrophenol and ouabain (Sigma Chemical Co., St. Louis, MO) were purchased.

Cell culture. Male Fischer 344 rats (Hilltop Laboratories, Scottdale, PA), 250–300 g, were deeply anesthetized with an overdose of pentobarbital and exsanguinated by severing the abdominal aorta. Alveolar macrophages were collected by pulmonary lavage under sterile conditions using eight 5-mL aliquots of warm, filtered calcium- and magnesium-free Hanks' Balanced Salt Solution (pH 7.4). The 40-mL samples were centrifuged at 3000 *g* for 7 min. The cell pellets containing alveolar macrophages were resuspended in sterile growth medium containing (per 100 mL) 80 mL RPMI 1640 with L-glutamine, 0.5 mL gentamicin sulfate (10 mg/mL), 0.5 mL 2-mercaptoethanol (10 mmol in 0.02 N KOH), and 10 mL fetal bovine serum. Cells were plated at a concentration of 1.0×10^6 cells/tissue culture well. Cell cultures were treated with amiodarone and desethylamiodarone alone or in combination (1, 2.5, or 5 μM). These concentrations were used since it has been shown that the therapeutic plasma concentration range in humans is 1–2 μg/mL. This concentration range is covered since the levels we used give concentrations of 0.65 to 3.2 μg/mL. Amiodarone and desethylamiodarone (2.5 μM) were incubated with various metabolic inhibitors [KCN (1 mM), 2,4-dinitrophenol (0.5 mM), and ouabain (1 mM)] or other cationic, amphiphilic drugs [imipramine (20 μM), chlorcyclizine (20 μM), and chlorphentermine (20 μM)]. Cells were incubated with drugs for varying periods of time at 37° in a tissue incubator containing air and 5% carbon dioxide.

Drug accumulation. Following incubation, the conditioned medium was removed from the plates of cell cultures treated as described above and centrifuged to remove floating cells. The adherent cells were washed gently with saline two times, and then were removed using trypsin and added to the floating cells. The cells were centrifuged, washed, and then resuspended in 0.5 mL of culture medium. Cells were then counted using a hematocytometer. Cell viability was measured by trypan blue exclusion and was greater than 90% for all experiments. Drug levels were measured in cells using HPLC as described by Reasor *et al.* [11].

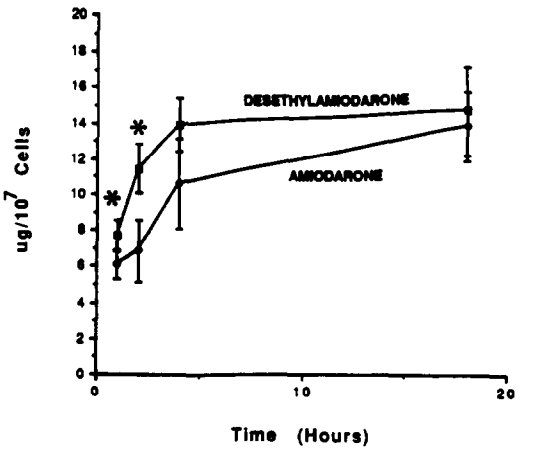

Fig. 1. Amiodarone and desethylamiodarone uptake by alveolar macrophages after 1-, 2-, 4-, and 18-hr incubations. A 2.5 μM concentration was used for each drug. Values are means ± SEM (N = 5). At 1 and 2 hr, the mean values of desethylamiodarone were significantly greater than amiodarone values (*$P < 0.05$).

Statistics. The comparisons between groups were made using ANOVA. The significance of difference between groups was tested using the Student–Newman–Keuls procedure [20]. The significance of difference between groups in the concentration–response experiments (see Fig. 2) was analyzed using two-way analysis of variance [20]. The significance of difference between amiodarone and desethylamiodarone was measured by paired *t*-test analysis [20]. The criterion used for significance was $P < 0.05$.

RESULTS

After 1 and 2 hr of incubation, the uptake of desethylamiodarone by alveolar macrophages was significantly greater than that of amiodarone at a concentration of 2.5 μM (Fig. 1). Over time, the accumulation of amiodarone by the cells began to approach that of desethylamiodarone and was not significantly different by 4 hr. Within this 4-hr time period, the accumulation of both of these drugs was nearly complete.

The incubation of alveolar macrophages with either amiodarone or desethylamiodarone at concentrations of 1, 2.5 or 5 μM for 1 hr displayed a linear, concentration–response relationship for both drugs (Fig. 2). At all three concentrations, there was a preferential uptake of desethylamiodarone by the alveolar macrophages as compared to amiodarone.

To examine whether the preferential uptake of desethylamiodarone occurred under more relevant pharmacological conditions, cells were incubated with both drugs together. Plasma levels measured in the adult male Fischer 344 rat after 1 week of amiodarone treatment (150 mg/kg) were used [15], giving a desethylamiodarone concentration of 0.8 μg/

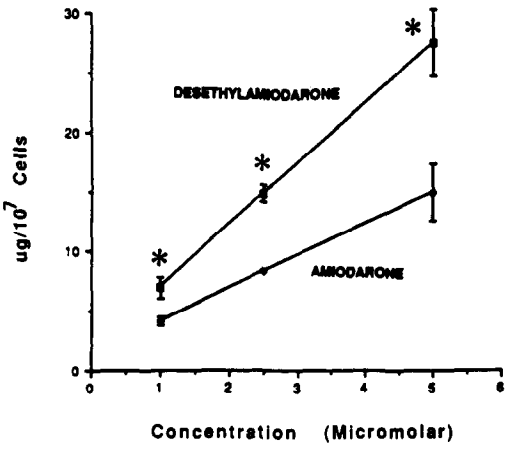

Fig. 2. Concentration–response of amiodarone and desethylamiodarone uptake by alveolar macrophages at concentrations of 1, 2.5 and 5 μM after a 1-hr incubation period. Values are means ± SEM (N = 6). Mean values for desethylamiodarone were significantly greater than amiodarone values (*P < 0.05).

mL and an amiodarone concentration of 1.95 μg/mL, and a desethylamiodarone to amiodarone ratio of 0.4. The proportional uptake of each drug was measured and compared to the ratio present in the culture medium. After both 1 and 18 hr of incubation, the ratio of desethylamiodarone to amiodarone uptake was significantly greater than the ratio present in the medium containing no cells, indicating an increased uptake of the desethylamiodarone by the alveolar macrophages under these conditions as compared to amiodarone (Fig. 3).

Metabolic inhibitors were used to investigate the possible transport mechanisms which may be involved in the accumulation of these drugs by the alveolar macrophage. Both amiodarone and desethylamiodarone were incubated separately for 1 hr with either KCN, 2.4-dinitrophenol, or ouabain. None of these inhibitors had any effect in blocking the uptake of these drugs into the alveolar macrophages (Fig. 4). In fact, the level of amiodarone was greater than control values when cells were co-treated with KCN or 2,4-dinitrophenol.

Amiodarone and desethylamiodarone efflux from alveolar macrophages was measured after each drug was incubated individually with the cells for 4 hr. Following this incubation period, the culture medium was removed, drug-free medium was added, and the cells were incubated for another 24 hr. Sixty-three percent of amiodarone was lost from the cells as compared to only 31% of desethylamiodarone during this 24-hr period (Fig. 5). It was possible that the efflux process was inhibited by KCN and 2,4-dinitrophenol resulting in the elevated accumulation of amiodarone in the cells (Fig. 4). To examine this question, cells were incubated for 4 hr with amiodarone, and then placed in drug-free medium containing either KCN or 2,4-dinitrophenol. Neither metabolic inhibitor affected the export of amiodarone from alveolar macrophages (Fig. 6).

To determine whether a carrier-mediated transport process was occurring, where competition may be demonstrated, amiodarone and desethylamiodarone were incubated separately for 1 hr with one of three cationic, amphiphilic drugs, either chlorcyclizine, chlorphentermine, or imipramine. None of these physicochemically similar drugs had any effect in preventing the accumulation of either amiodarone or desethylamiodarone by the cells (data not shown).

DISCUSSION

It has been well documented that amiodarone and desethylamiodarone accumulate in alveolar macrophages and whole lung tissue in both humans [12, 16] and animals [11, 15, 21, 22]. When rats were treated with amiodarone, alveolar macrophages

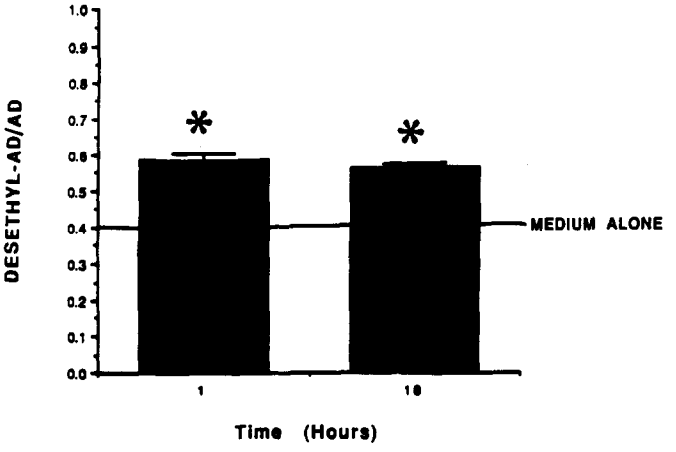

Fig. 3. Desethylamiodarone (Desethyl-AD) to amiodarone (AD) ratio of uptake by alveolar macrophages after 1- and 18-hr incubations. A desethylamiodarone concentration of 0.80 μg/mL and an amiodarone concentration of 1.95 μg/mL were used. Values are means ± SEM (N = 5). Mean values of the desethyl-AD/AD ratio were significantly greater than ratios of media alone (*P < 0.05).

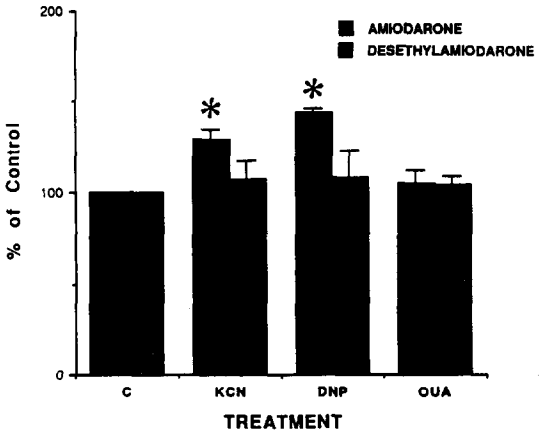

Fig. 4. Amiodarone and desethylamiodarone uptake by alveolar macrophages when incubated with either KCN, 2,4-dinitrophenol (DNP), or ouabain (OUA) for 1 hr. Uptake was measured as $\mu g/10^7$ cells and displayed as percent of control (amiodarone: $4.86 \pm 0.29\ \mu g/10^7$ cells and desethylamiodarone: $7.41 \pm 0.84\ \mu g/10^7$ cells). A 2.5 μM concentration of amiodarone or desethylamiodarone was used with inhibitor concentrations of 0.5 mM (DNP) or 1 mM (KCN and OUA). Values are means ± SEM (N = 3). Mean values of amiodarone + KCN and amiodarone + DNP were significantly greater than controls (*$P < 0.05$).

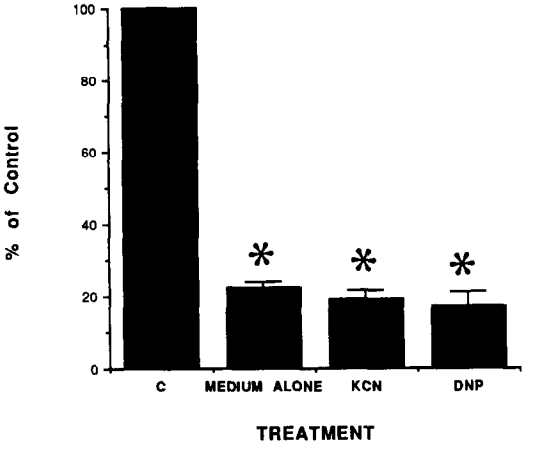

Fig. 6. Amiodarone efflux from alveolar macrophages over 24 hr when incubated with either KCN or 2,4-dinitrophenol (DNP). Uptake was measured as $\mu g/10^7$ cells and displayed as percent of control ($11.21 \pm 0.90\ \mu g/10^7$ cells). Cells were incubated with 2.5 μM amiodarone for 4 hr, then placed in drug-free medium containing no additions, KCN (1 mM), or DNP (0.5 mM) and incubated for another 24 hr. Values are means ± SEM (N = 3). Mean values of medium alone, amiodarone + KCN, and amiodarone + DNP were significantly less than control values (*$P < 0.05$).

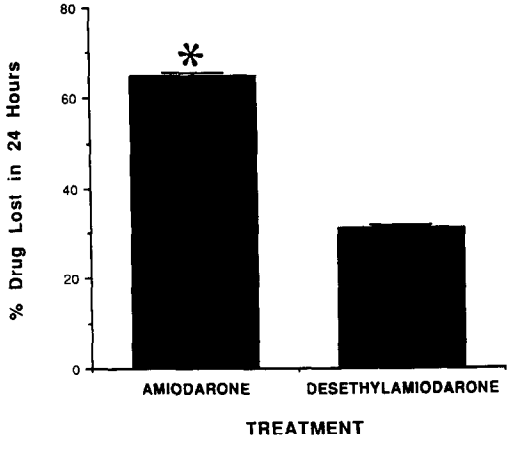

Fig. 5. Amiodarone and desethylamiodarone efflux from alveolar macrophages over 24 hr. Cells were incubated separately with 2.5 μM concentrations of amiodarone or desethylamiodarone. Control values for amiodarone and desethylamiodarone were 10.19 ± 1.54 and $14.54 \pm 0.82\ \mu g/10^7$ cells, respectively. Values are means ± SEM (N = 3). Mean values of percent amiodarone lost were significantly greater than those of percent desethylamiodarone lost (*$P < 0.05$).

showed a greater proportional increase in the accumulation of amiodarone, desethylamiodarone, and total phospholipid than did lung parenchymal tissue, acellular lavage material, or type II cells [17].

Metabolism of amiodarone appears not to occur in rat alveolar macrophages [18] or rat lung [22, 23]. Thus, it was possible to study the accumulation of amiodarone and desethylamiodarone individually by alveolar macrophages without the complication of metabolism. In this study, we demonstrated *in vitro* that both amiodarone and desethylamiodarone accumulate within the alveolar macrophages. This accumulation exhibited a linear, concentration-dependent response for both drugs. Our results are suggestive of a preferential uptake of the metabolite, desethylamiodarone, as compared to amiodarone in the alveolar macrophages. These findings are not surprising since it has been shown that compared to amiodarone, desethylamiodarone accumulates to a significantly higher level in alveolar macrophages following administration of amiodarone to rats and humans [11, 12]. Using X-ray microanalysis, we have reported previously that both drugs accumulate within the cells and are not just bound to the plasma membrane [24]. These drugs were shown to accumulate in the amorphous bodies and dense granules, structures representative of the lysosomal compartment.

To characterize the transport mechanism involved in the uptake of amiodarone and desethylamiodarone by the alveolar macrophages, the drugs were incubated with a variety of metabolic inhibitors. Potassium cyanide (an inhibitor of the last step of the mitochondrial electron transport chain), 2,4-dinitrophenol (an uncoupler of electron transport), and ouabain (Na^+-K^+-ATPase inhibitor) were used. Amiodarone and desethylamiodarone uptake was not blocked by the inhibitors nor showed a dependency for the Na^+-K^+-ATPase pump. This finding indicated the lack of involvement of an active process, but did not disprove the possibility of a

facilitated component of drug uptake by the alveolar macrophages.

An interesting observation was seen with our experiments using the metabolic inhibitors. Upon treatment of alveolar macrophages with amiodarone and KCN or 2,4-dinitrophenol, we observed an increase in the uptake of amiodarone by the cells compared to the control. The basis for this increased accumulation of the drugs under these conditions is unclear at present. Although speculative, the membrane structure of the alveolar macrophage may be distorted by the high concentration of the metabolic inhibitors allowing more drug to enter the cells, or it may be an impairment of the export mechanism of the cell. We performed studies on the efflux of amiodarone from alveolar macrophages in the presence of KCN or 2,4-dinitrophenol. These metabolic inhibitors had no effect in altering the export of amiodarone from the cell. Additional studies would have to be done to determine if these inhibitors are causing membrane structure perturbation of the alveolar macrophage.

We have provided evidence previously that the accumulation of another cationic, amphiphilic drug, chlorphentermine, is carrier-mediated at low concentrations and is inhibited by other structurally similar drugs [25]. Therefore, other cationic, amphiphilic drugs (imipramine, chlorcyclizine, and chlorphentermine) were used to determine whether a carrier-mediated competition could be demonstrated in the movement of amiodarone and desethylamiodarone into alveolar macrophages. It appears that no competition is occurring since the accumulation of amiodarone and desethylamiodarone was not altered when incubated together with chemically similar drugs. Our results suggest that passive diffusion may be involved in the transport of these drugs into the alveolar macrophages.

This passive mechanism of transport was not unexpected. Amiodarone and desethylamiodarone are cationic, amphiphilic drugs which contain a hydrophobic ring structure, a hydrophilic side chain, and substituted nitrogen which is charged at physiological pH. These drugs interact with negatively charged and neutral polar lipids which regulate the entry and binding of these drugs in cells [26]. Amiodarone has a low water solubility and a high lipid to water partition coefficient, and displays significant hydrophobic behavior [27]. This drug alters lipid dynamics and the physiological state of normal membranes at micromolar concentrations [28]. Fluorescence polarization [29] and fluorescence-binding studies [30, 31] of amiodarone with lipids have indicated that amiodarone partitions into the hydrophobic core of the lipid bilayer. Due to structural similarities, it seems likely that desethylamiodarone would display the same properties as amiodarone.

The efflux of amiodarone and desethylamiodarone from alveolar macrophages was measured over a 24-hr period. We found a 63% loss of amiodarone from the cells as compared to a 31% loss of desethylamiodarone. These results indicated an enhanced retention of desethylamiodarone by the alveolar macrophages as compared to amiodarone. This then suggests that desethylamiodarone may remain more avidly associated with the alveolar macrophage as compared to amiodarone. These results are consistent with those observed in rats *in vivo* following withdrawal from amiodarone treatment [11].

Many studies have shown an increase in cytotoxicity of desethylamiodarone as compared to amiodarone. This has been demonstrated in cell cultures of thyrocytes [32], fibroblasts [32], hepatocytes [33, 34], and alveolar macrophages [18]. Desethylamiodarone was found to be slightly more potent than amiodarone in inhibiting rat lung lysosomal phospholipase A_1 [35]. Both drugs are capable of inducing the formation of lamellar inclusions *in vitro*, with desethylamiodarone appearing to be more effective than amiodarone [18]. In addition, it has been reported that desethylamiodarone is more potent than amiodarone in inducing pulmonary fibrosis when administered by intratracheal instillation [19].

In this investigation, we have demonstrated a difference in the alveolar macrophage uptake and retention of these two compounds. Our observations may provide a plausible explanation for the different pulmonary responses seen with amiodarone and desethylamiodarone. The increased accumulation and retention of desethylamiodarone may provide a foundation for further experimentation in trying to solve the problem of amiodarone-induced pulmonary injury. For now, the mechanism of this toxicity remains unclear.

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REFERENCES

1. Mason JW, Amiodarone. *N Engl J Med* **316**: 455–465, 1987.
2. Akoun GM, Liote G, Milleron BJ and Maynard CM, Amiodarone lung. *Current Topics in Pulmonary Pharmacology and Toxicology* (Ed. Hollinger MA). Elsevier Science Publishing, New York, 1987.
3. Sobol SM and Rakita L, Pneumonitis and pulmonary fibrosis associated with amiodarone treatment: A possible complication of a new antiarrhythmic drug. *Circulation* **65**: 819–824, 1982.
4. Marchlinski FE, Gansler TS, Waxman HL and Josephson ME, Amiodarone pulmonary toxicity. *Ann Intern Med* **97**: 839–845, 1982.
5. Suarez LD, Poderoso JJ, Elsner B, Bunster AM, Esteva H and Bellotti M, Subacute pneumopathy during amiodarone therapy. *Chest* **83**: 566–568, 1983.
6. Fan K, Bell, R, Eudy S and Fullenwider J, Amiodarone-associated pulmonary fibrosis: Evidence of an immunologically-mediated mechanism. *Chest* **92**: 625–630, 1987.
7. Liu FL, Cohen RD, Downar E, Butany JW, Edelson JD and Rebuck AS, Amiodarone pulmonary toxicity: Functional and ultrastructural evaluation. *Thorax* **41**: 100–105, 1986.
8. Kennedy JI, Myers JL, Plumb VJ and Fulmer JD, Amiodarone pulmonary toxicity: Clinical, radiologic and pathologic correlations. *Arch Intern Med* **147**: 50–55, 1987.

9. Adams PC, Gibson GJ, Morley AR, Wright AJ, Corris PA, Reid DS and Campbell RF, Amiodarone pulmonary toxicity: Clinical and subclinical features. *Q J Med New Series* **59**: 449–471, 1986.
10. Dean PJ, Groshart KD, Porterfield JG, Iansmith DH and Golden EB, Amiodarone-associated pulmonary toxicity. A clinical and pathological study of eleven cases. *Am J Clin Pathol* **87**: 7–13, 1987.
11. Reasor MJ, Ogle CL, Walker ER and Kacew S, Amiodarone-induced phospholipidosis in rat alveolar macrophages. *Am Rev Respir Dis* **137**: 510–518, 1988.
12. Martin WJ II and 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.
13. Myers JL, Kennedy JI and Plumb VJ, Amiodarone lung: Pathologic findings in clinically toxic patients. *Hum Pathol* **18**: 349–354, 1987.
14. Colgan T, Simon GT, Kay JM, Pugsley SO and Eydt J, Amiodarone pulmonary toxicity. *Ultrastruct Pathol* **6**: 199–207, 1984.
15. Reasor MJ, Ogle CL and Kacew S, Amiodarone-induced pulmonary toxicity in rats: Biochemical and pharmacological characteristics. *Toxicol Appl Pharmacol* **97**: 124–133, 1989.
16. Brien JF, Jimmo S, Brennan FJ, Ford SE and Armstrong PW, Distribution of amiodarone and its metabolite, desethylamiodarone, in human tissues. *Can J Physiol Pharmacol* **65**: 360–364, 1987.
17. Reasor MJ, Ogle CL and Miles PR, Response of rat lungs to amiodarone: Preferential accumulation of amiodarone and desethylamiodarone in alveolar macrophages. *Exp Lung Res* **16**: 577–591, 1990.
18. Ogle CL and Reasor MJ, Response of alveolar macrophages to amiodarone and desethylamiodarone, *in vitro*. *Toxicology* **62**: 227–238, 1990.
19. Daniels JM, Brien JM and Massey TE, Pulmonary fibrosis in the hamster by amiodarone and desethylamiodarone. *Toxicol Appl Pharmacol* **100**: 350–356, 1989.
20. Tallarida RJ and Murray RB, *Manual of Pharmacological Calculations with Computer Programs*. Springer, New York, 1981.
21. Kannan R, Sarma JM, Guha M and Venkataraman K, Tissue drug accumulation and ultrastructural changes during amiodarone administration in rats. *Fundam Appl Toxicol* **13**: 793–803, 1989.
22. Camus P and Mehendale HM, Pulmonary sequestration of amiodarone and desethylamiodarone. *J Pharmacol Exp Ther* **237**: 867–873, 1986.
23. Young RA and Mehendale HH, *In vitro* metabolism of amiodarone by rabbit and rat liver and small intestine. *Drug Metab Dispos* **14**: 423–431, 1986.
24. Reasor MJ, Lee P and Kirk RG, Application of X-ray microanalysis to the study of drug uptake in cell culture. *Exp Mol Pathol* **53**: 64–71, 1990.
25. Heyneman CA and Reasor MJ, Role of the alveolar macrophage in the induction of pulmonary phospholipidosis by chlorphentermine. II. Drug uptake into cells *in vitro*. *J Pharmacol Exp Ther* **236**: 60–64, 1986.
26. Kodavanti UP and Mehendale HM, Cationic amphiphilic drugs and phospholipid storage disorder. *Pharmacol Rev* **42**: 327–354, 1990.
27. Warren RJ, Stedman RJ, Shami EG, Rattie ES and Ravin LJ, Observations on the micelle formation of 2-butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride (SK&F 33134-A) by NMR spectroscopy. *J Pharm Sci* **59**: 1357–1358, 1970.
28. Chatelain P and Brotelle R, Phospholipid composition of rat lung after amiodarone treatment. *Res Commun Chem Pathol Pharmacol* **50**: 407–418, 1985.
29. Chatelain P, Ferreira J, Laurel R and Ruysschaert JM, Amiodarone-induced modifications of the phospholipid physical state. A fluorescence polarization study. *Biochem Pharmacol* **35**: 3007–3013, 1986.
30. Joshi UM, Kodavanti PR, Coudert B, Dwyer TM and Mehendale HM, Types of interaction of amphiphilic drugs with phospholipid vesicles. *J Pharmacol Exp Ther* **246**: 150–157, 1988.
31. Joshi UM, Kodavanti PR, Lockard VG and Mehendale HM, Fluorescence studies on binding of amphiphilic drugs to isolated lamellar bodies. *Biochim Biophys Acta* **1004**: 309–320, 1989.
32. Beddows SA, Page SR, Taylor AH, McNerney R, Withley GS, Johnstone AP and Nussey SS, Cytotoxic effects of amiodarone and desethylamiodarone on human thyrocytes. *Biochem Pharmacol* **38**: 4391–4403, 1989.
33. Gross SA, Bandyopadhyay S, Klaunig JE and Somani P, Amiodarone and desethylamiodarone toxicity in isolated hepatocytes in culture. *Proc Soc Exp Biol Med* **190**: 163–169, 1989.
34. Bandyopadhyay S and Somani P, A comparison of plasma, white blood cells, red blood cell and tissue distribution of amiodarone and desethylamiodarone in anesthetized dogs. *J Cardiovasc Pharmacol* **10**: 379–388, 1987.
35. Hostetler KY, Giordano JR and Jellison EJ, *In vitro* inhibition of lysosomal phospholipase A_1 of rat lung by amiodarone and desethylamiodarone. *Biochim Biophys Acta* **959**: 316–321, 1988.