MINIREVIEW

An Evaluation of Possible Mechanisms Underlying Amiodarone-Induced Pulmonary Toxicity[1] (44019)

MARK J. REASOR*,[2] AND SAM KACEW†

Department of Pharmacology and Toxicology, Robert C. Byrd Health Sciences Center of West Virginia University, Morgantown, West Virginia 26506-9223; and Department of Pharmacology,† University of Ottawa, Ottawa, Ontario, K1H 8M5 Canada*

Abstract. **The effectiveness of amiodarone in the treatment of cardiac arrhythmias is limited due to the development of pulmonary toxicity. Although the biochemical and morphologic characteristics associated with amiodarone-induced pulmonary toxicity (AIPT) are well-defined, the mechanisms underlying this disorder remain unknown. This review focuses on proposed mechanisms of AIPT, in particular (i) direct cellular damage; (ii) the role of phospholipidosis; (iii) the correlation between drug burden and toxicity; (iv) the role of the immune system; (v) the generation of oxidants; (vi) changes in membrane properties; and (vii) miscellaneous biochemical considerations. Additional discussion of the role of amiodarone's primary metabolite, desethylamiodarone, in AIPT and the involvement of preexisting lung dysfunction in the susceptibility to AIPT is included. With a clearer understanding of the possible contributions of these mechanisms to AIPT, it may be possible to develop strategies to alleviate toxicity and prolong the usefulness of amiodarone in the treatment of cardiac arrhythmias.** [P.S.E.B.M. 1996, Vol 212]

Amiodarone is a benzofuran derivative with class III antiarrhythmic activity (Fig. 1). It has potent suppressive activity on supraventricular and ventricular cardiac arrhythmias; however, numerous side effects limit its utility (1, 2). Pulmonary toxicity is perhaps the most significant and potentially life-threatening side effect associated with amiodarone use (3–5). The incidence of amiodarone-induced pulmonary toxicity (AIPT) has been reported to be approximately 6%, with a mortality rate estimated at 5%–10% of affected patients (5).

Pathology

AIPT is characterized pathologically by the large and abnormal accumulation of phospholipids in the lungs (i.e., development of a phospholipidosis), interstitial and intra-alveolar inflammation (alveolitis), with fibrosis developing in some patients (3). The principal phospholipidotic response is the appearance of large intraalveolar cells which have a "foamy" appearance under the light microscope. These cells, which appear to originate from alveolar macrophages, contain lysosomally derived lamellar inclusions (6, 7) visible with the electron microscope. Other pulmonary cell types, including endothelial cells (8), interstitial cells (9),

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[2] To whom requests for reprints should be addressed at Department of Pharmacology and Toxicology, Robert C. Byrd Health Sciences Center of West Virginia University, P.O. Box 9223, Morgantown, WV 26506-9223.

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bronchiolar epithelial cells (8), and type II cells (9, 10), may contain the lamellar inclusions. The inflammatory response produced by amiodarone is manifested by thickening of alveolar walls and variable infiltration by plasma cells, lymphocytes, eosinophils, and neutrophils (3, 7). Inflammatory cells can be recovered by bronchoalveolar lavage (11, 12). Edema (7, 13–15), and an intra-alveolar fibrin exudate (14) may accompany the inflammatory response. Amiodarone-induced fibrosis may be patchy and interstitial and/or intra-alveolar (16–20). A case of acute necrotizing pneumonitis associated with amiodarone administration has been described (21).

Experimental Studies

In an attempt to study features of AIPT under more controlled conditions, a large number of publications have appeared in which laboratory animals have been used. While pulmonary phospholipidosis is produced consistently in animals using oral administration of amiodarone (22–25), inflammation and fibrosis are not. Wilson *et al.* (24) and Vereckei *et al.* (26) reported that amiodarone induced pulmonary inflammation in rats, while Reasor *et al.* (22) did not find this response. The inflammatory response occurring with oral administration appears to occur only at rather high doses of amiodarone (24, 26). There is only one report of which we are aware where both alveolitis and fibrosis appear to result from the oral administration of amiodarone (26). Others have not found oral amiodarone administration to cause fibrosis in animals (27–29).

If amiodarone is administered to animals by the intratracheal route, however, alveolitis and fibrosis, similar in histopathology to AIPT in humans, can be induced (29–31). As a result, this is generally the administration route of choice for the induction of AIPT in animals.

Despite a large literature of AIPT pathology, little information exists on the mechanism(s) responsible for this disorder. In recent years, a number of review articles have been published describing features associated with AIPT (4, 6, 32–35). It has been several years since possible mechanisms of AIPT have been reviewed, and during that time a number of studies relevant to this question have appeared. Since amiodarone is an important drug in the treatment of certain arrhythmias, and its use will continue to increase, it is necessary to understand the etiology of AIPT as completely as possible (36). The purpose of the present review is to provide an update of our understanding of the etiology of AIPT.

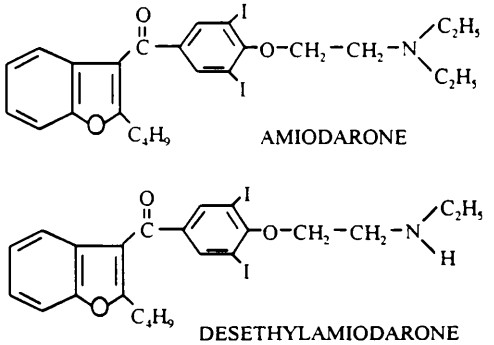

Figure 1. Structure of amiodarone and its primary metabolite, desethylamiodarone.

Possible Mechanisms Involved in AIPT

AIPT may present in several ways, suggesting that there is more than one mechanism for its etiology (33). As a result, a number of theories have been set forth to explain the development of AIPT, including (i) direct cellular damage, (ii) the development of phospholipidosis, (iii) the level of drug in the tissue (drug burden), (iv) immunological mechanisms, (v) oxidative mechanisms, and (vi) changes in membrane properties. The evidence from human and animals studies supporting these theories will be reviewed and evaluated.

Direct Cellular Toxicity. Amiodarone and its primary metabolite, desethylamiodarone (Fig. 1), are cytotoxic when incubated with various cell types *in vitro*. Data from a study by Martin and Howard (37) were the first to suggest a role of direct cellular damage as a possible mechanism for AIPT. Amiodarone was cytotoxic to bovine pulmonary artery endothelial cells after 18 hr in cell culture as monitored by ^{51}Cr release. Subsequently, Ogle and Reasor (38) reported that amiodarone and desethylamiodarone were cytotoxic to rat alveolar macrophages at 18 hr in cell culture as assessed by the release of lactate dehydrogenase (LDH). Desethylamiodarone was more cytotoxic than amiodarone at equal concentrations. Interestingly, exposure of cells to both amiodarone and desethylamiodarone resulted in a LDH release at 18 hr that was greater than the sum of the cytotoxicities of drugs individually. Wilson and Lippmann (39) demonstrated that both drug species were cytotoxic to bovine pulmonary artery cells and rabbit lung fibroblasts over 24 hr in culture. The desethylamiodarone metabolite was more cytotoxic than amiodarone to both cell types. While amiodarone and its metabolite are capable of killing cells, it is not clear as to the exact role this property would play in initiating AIPT. Additional work assessing the possible release of inflammatory and/or fibrogenic mediators associated with cytotoxicity would be needed before a direct relationship could be considered.

Phospholipidosis. Humans afflicted with AIPT invariably demonstrate phospholipidosis in "foamy" alveolar macrophages, giving rise to the hypothesis that a cause-and-effect relationship may exist between the two conditions. It is widely believed that the mech-

anism by which amiodarone induces the phospholipidosis develops is by inhibition of lysosomal phospholipase activity in the lungs, which leads, in part, if not totally, to the marked accumulation of phospholipid in the lungs (33, 34). Both amiodarone and desethylamiodarone are potent inhibitors of pulmonary lysosomal phospholipase activity in animals (40–44).

Camus and Jeannin (45) speculated that the derangement in lipid metabolism associated with the induction of phospholipidosis may be associated with the pulmonary toxicity of amiodarone. While the inhibition of phospholipid catabolism appears to be responsible for the phospholipidosis component of AIPT, there is no consistent evidence that the induction of phospholipidosis *per se* is causally related to the development of AIPT. There are reports of patients receiving amiodarone who have "foamy" macrophages but do not have evidence of clinical toxicity of AIPT (15, 20, 46–48). Although this suggests that phospholipidosis is not a mechanism involved in AIPT, it cannot be ruled out as contributing to this disorder. It is possible that patients with phospholipidosis but without AIPT described in these reports had not yet progressed to a toxic condition.

Blake and Reasor (29) addressed the question of the involvement of phospholipidosis in the induction of pulmonary fibrosis in hamsters by amiodarone. Hamsters were given amiodarone either orally daily (5 days/week) or by intratracheal administration on Day 0 and 7, and the presence of phospholipidosis and pulmonary fibrosis assessed on Day 28. Pulmonary phospholipidosis developed when amiodarone was administered orally but not intratracheally. In contrast, pulmonary fibrosis developed following intratracheal amiodarone but not oral amiodarone. This study indicated that phospholipidosis was not a factor in the development of AIPT in hamsters.

Drug Burden. One characteristic of AIPT is the substantial accumulation of amiodarone and desethylamiodarone in lung tissue in humans (6, 13, 49, 50). The same accumulation occurs in animals in association with the development of amiodarone-induced phospholipidosis (23, 24, 51). Lung tissue to plasma ratios up to 1600 have been reported for the combined level of amiodarone and desethylamiodarone in humans (50) and animals (51–53) with values dependent on the dosage and duration of treatment. In some studies, the incidence of AIPT has been correlated with the duration of treatment, suggesting that the development of the disorder is a function of the accumulation of amiodarone and desethylamiodarone in the lungs (45) or of the drug dosage (9, 54). Other reports do not support a direct relationship between average daily dose or cumulative total dose of amiodarone and the occurrence of AIPT (13, 14, 46, 48, 50). Interestingly, once AIPT developed impairments in pulmonary function were correlated with drug dose and duration of treatment (46).

In the study by Blake and Reasor (29) described earlier, drug levels were measured in hamster lungs following oral administration of amiodarone where fibrosis did not develop and following intratracheal administration of amiodarone where this response occurred. As a result of oral administration, levels of amiodarone and desethylamiodarone were substantial at Day 28, while neither drug species was detectable beyond Day 10 following intratracheal administration. On Day 8 of both protocols, which was the day after the second intratracheal instillation, the combined level of amiodarone and desethylamiodarone was approximately 50-fold higher in the orally treated animals. From this study, it can be concluded that in the hamster model, the development of AIPT is not related to the amount of amiodarone and/or desethylamiodarone in the lung.

Immunological Mechanisms. The theory has been put forth that immunopathological mechanisms contribute to AIPT, although this is not a universal finding among patients (5, 33). There is evidence that the response may be cell-mediated or humoral (11, 55) with features similar to hypersensitivity pneumonitis (56–58). The number of reports is too great to permit individual discussion of each; therefore, studies supporting and refuting immunopathological involvement are listed in Table I. The absence of immunopathological processes in some patients may be due to the fact that this mechanism was involved but may not have been detectable at the time of assessment.

From limited animal studies, however, it appears that amiodarone is capable of affecting certain immune-mediated processes. Karpel *et al.* (63) reported an activation of natural killer cell activity in cells recovered from minced rat lungs following amiodarone administration. Alveolar macrophages from rats treated with amiodarone show inconsistent alterations in cytokine secretion *in vitro*. Wilson *et al.* (64) reported that interleukin-1 secretion is augmented from lipopolysaccharide-stimulated alveolar macrophages, while Reasor *et al.* (65) found no effect on this cytokine; however, lipopolysaccharide-stimulated alveolar macrophages secreted elevated levels of interleukin-6 and tumor necrosis factor–α *in vitro*. Exactly how these alterations may contribute to AIPT is unclear at present. Based upon the inconsistent evidence for the presence of abnormal immune processes in humans with AIPT, it appears that immune-mediated alterations may be contributory to AIPT in some individuals but are not a predominant mechanism.

Oxidative Mechanisms. Based on a number of animal experiments, evidence is accumulating that the development of AIPT may involve augmented oxidative processes in the lung. Most of the studies utilized

Table I. Evidence for and against Immunopathological Responses in Amiodarone-Pulmonary Toxicity

Response	Reference
Evidence favoring a role for immunopathological processes	
Deposition of C3 fragment of complement	58, 59
Deposition of IgG	59
Bronchoalveolar fluid lymphocytosis	11, 55, 58, 60, 61
Bronchoalveolar fluid with a reversed ratio of CD^+:$CD8^+$ lymphocytes	11, 55, 58, 61
Bronchoalveolar fluid eosinophilia	11, 59, 60
Positive lymphoblastic transformation in presence of amiodarone	55
Positive basophil degranulation/test in presence of amiodarone	55
Secretion of leukocyte inhibitory factor in presence of amiodarone	55
Antibodies in serum of patient with AIPT that reacted with patient's lung tissue	56
Evidence against a role for immunopathological processes	
Absence of deposition of C3 fragments and immunoglobulins	17
Absence of lymphocytosis	46, 62
Absence of deposition of immunoglobulins or complement	20
No difference in basophil degranulation or lymphoblastic transformation tests between patients with or without AIPT	48
Presence and absence of anti-amiodarone antibodies in serum of a group of patients with AIPT	57
Lack of evidence of immune or autoimmune processes	13

a regimen of antioxidants; in some studies, antioxidant therapy was successful in blunting AIPT and in others it was not. Kennedy *et al.* (66) were perhaps the first to implicate oxidants in acute amiodarone-induced lung damage using ventilated and perfused rabbit lungs. Perfusion with amiodarone resulted in the development of edema associated with increases in cyclooxygenase and lipoxygenase metabolites in the perfusate. Additionally, perfusion with amiodarone resulted in an increased production of superoxide anion and enhanced whole lung chemiluminescence. The level of tissue reduced glutathione was increased significantly after perfusion with amiodarone. Addition of the combined cyclooxygenase and lipoxygenase inhibitor enolicam sodium or catalase or superoxide dismutase plus catalase to the perfuse did not protect against the formation of pulmonary edema. In contrast, pretreatment with butylated hydroxyanisole, vitamin E, or N-acetylcysteine or ventilation with 4% oxygen protected against amiodarone-induced edema. While oxidant-released processes appear capable of causing acute pulmonary edema, the relationship of this injury to the alveolitis and fibrosis occurring with chronic amiodarone treatment is unclear.

Using human pulmonary artery endothelial cells in cell culture, Kachel *et al.* (67) were unable to alter amiodarone-induced cytotoxicity with hypoxia or hyperoxia or prevent damage with the antioxidants catalase, superoxide dismutase, ascorbic acid, dimethyl sulfoxide, or ethanol. In contrast, vitamin E prevented damage from occurring; however, it did not inhibit the uptake of amiodarone into the cells. In light of the ineffectiveness of other antioxidants to offer protection, vitamin E appears to be effective through a mechanism other than inhibiting oxidation.

Recent studies on the effects of vitamin E on cultured skin fibroblasts demonstrated that vitamin E can reduce the induction of phospholipidosis by amiodarone or desethylamiodarone (68). Vitamin E also accelerated the clearance of previously accumulated phospholipid after discontinuation of drug treatment. Although the results are not in cells from the lungs, they support the idea that vitamin E can effectively attenuate amiodarone toxicity. The results of this study and that of Kachel *et al.* (67) suggest that the actions of vitamin E may be related to actions on the cell membrane (69, 70).

Vereckei *et al.* (26) reported that treatment with the antioxidant silibinin reduced the extent of phospholipidosis induced in rat lung by amiodarone. MTDQ-DA, a dihydroquinolin-type antioxidant, was not protective. Neither antioxidant was effective in preventing AIPT. The authors proposed that free radicals are involved in the induction of phospholipidosis.

A single intratracheal administration of amiodarone to hamsters induced pulmonary phospholipidosis and fibrosis accompanied by increases in tissue superoxide dismutase and lipid peroxidation (25). Dietary administration of niacin and/or taurine attenuated the phospholipidotic and fibrotic responses and inhibited the elevations in superoxide dismutase activity and lipid peroxidation. These data are consistent with the involvement of reactive oxygen species in amiodarone-induced lung damage in the hamster model.

The results of another study further implicate oxidative stress in the development of AIPT in hamsters. The single intratracheal administration of amiodarone to hamsters caused AIPT accompanied by an increased ratio of oxidized to total glutathione, and in-

creased activities of glutathione reductase, glutathione peroxidase, and superoxide dismutase (71), all endogenous antioxidant components. Pretreatment of the animals with either butylated hydroxyanisole, diallyl sulfide, or N-aceylcysteine failed to protect against AIPT. Since the antioxidants were not administered after amiodarone (i.e., during the time AIPT would be developing), it is not surprising that they were ineffective.

Zitnik *et al.* (72) reported that the phorbol myristate acetate-induced release of superoxide anion was enhanced from control rat alveolar macrophages exposed to amiodarone *in vitro,* providing evidence that the drug was capable of priming the cells to release this reactive oxygen species. In contrast, Reasor *et al.* (65) found that the luminol-dependent chemiluminescence of phospholipidotic alveolar macrophages from amiodarone-treated rats was not different compared with cells from control rats. Chemiluminescence is a measure of reactive oxygen release. In an approach examining the properties of the drug molecule, Vereckei *et al.* (26), demonstrated that amiodarone was capable of directly and indirectly generating free radicals. It was postulated that a very reactive aryl radical is formed after the metabolic partial deiodination of the drug molecule. This would provide a molecular explanation for amiodarone-mediated generation of free radicals that could participate in the development of AIPT. Deiodination of amiodarone by ultraviolet irradiation results in a free radical that is implicated in the peroxidation of lipids *in vitro* (73) providing indirect support for this idea. Alternatively, Kennedy *et al.* (66) speculate that metabolism of amiodarone to a free radical species might result in the formation of a hapten which could bind to macromolecules and initiate immunopathological reactions as discussed earlier.

While there is indication that AIPT may be mediated by oxidant mechanisms in cell culture and animal systems, there is no corroborating evidence in humans. Nevertheless, this is an attractive hypothesis that warrants further investigation.

Changes in Membrane Properties. A number of studies have revealed that amiodarone can interact with cellular membranes or artificial phospholipid vesicles/bilayers (74–81). Depending on the system studied the effects varied. Amiodarone increases membrane fluidity in human skin fibroblast plasma membranes (81), or decreases lipid fluidity in rat synaptic membranes, (74) and rat cardiac sarcolemmel vesicles (76). The effects on membrane fluidity from lung cells have not been studied to our knowledge. The molecular aspects of the membrane effects of the cationic, amphiphilic class of drugs of which amiodarone belongs is reviewed by Kodavanti and Mehendale (82). The exact relationship between membrane binding and perturbation by amiodarone and development of AIPT is unknown at present.

Other Considerations

In addition to the possible mechanisms discussed above, amiodarone can alter certain biochemical and cellular processes, and these activities may contribute to AIPT. These actions are summarized in Table II.

Reports have indicated that patients with preexisting lung dysfunction have a greater risk for the development of AIPT (15, 20, 87, 88). Others have not found this to be the case (89). If a preexisting lung disorder is present in patients developing AIPT, a common mechanism underlying AIPT probably does not exist.

The Role of Desethylamiodarone and Metabolism of Amiodarone in AIPT

Desethylamiodarone, the major metabolite of amiodarone, has been shown to accumulate in lung tissue to higher levels than amiodarone in humans (6, 50) and animals (39, 51) and is intrinsically more cytotoxic (38, 39), phospholipogenic (38), and fibrogenic (31) than amiodarone. Since both amiodarone and desethylamiodarone are present together in tissue, it is difficult to evaluate the relative contributions of each species to AIPT. Nevertheless, it is probable that desethylamiodarone plays a significant role in the development of AIPT.

It is of interest that F344 rats are much more susceptible to the development of amiodarone-induced pulmonary phospholipidosis than Sprague-Dawley rats, indicating a population-specific response (22, 44). Ratios of desethylamiodarone to amiodarone in plasma and lung tissue were greater in the F344 rats

Table II. Alterations in Biochemical and Cellular Activities by Amiodarone

Activity affected by amiodarone	Reference
Intracellular Ca^{2+} levels rise in human pulmonary artery cells in culture and also disrupted intracellular calcium homeostasis. The overall changes in calcium homeostasis correlated with an increase in cytotoxicity.	83
Activation of G proteins in HL60 cells.The drug's toxicity may be mediated through G protein activation.	84
Inhibition of rat pulmonary Na^+,K^+-ATPase which could be associated with the development of edema in some patients	51
Phospholipase C activity was inhibited in splenic white cells from rats. This led to the postulate that inhibition of cell signalling may be a mechanism for the inhibition of cellular functions	85
Phospholipase C-mediated release of free fatty acids from bovine artery endothelial cells at concentrations inducing cytotoxicity. Inflammatory fatty acids may participate in induction of AIPT.	86

than in Sprague-Dawleys consistent with differences in drug disposition, including metabolism, between species. It is possible that the susceptibility of certain humans and not others to AIPT is in like manner related to dispositional factors.

Conclusions

AIPT continues to be a problem with patients treated with amiodarone for cardiac arrhythmias. Despite a large number of case studies on patients and experimental studies in animals, the mechanism(s) by which AIPT develops is unknown.

Several theories have been proposed, however, either the data are inconsistent in humans or the results of animal studies have not been reported in humans. It would seem logical that nonspecific direct cellular damage (e.g., cytotoxicity) alone is insufficient to cause AIPT. Amiodarone interacts strongly with biological membranes; therefore, it would not be surprising if certain aspects of membrane function were affected in a way that results in cellular toxicity. The protective effect of the membrane-stabilizing agent, vitamin E, is evidence for this concept. Alterations in calcium homeostasis may be involved in initiating AIPT. The lack of a clear-cut relationship between average daily dose or cumulative daily dose and the development of AIPT suggests factors other than the direct action of amiodarone are responsible for AIPT. The finding that desethylamiodarone is capable of inducing AIPT in animals suggests that the metabolism of amiodarone may play an essential role in the development of AIPT.

The presence of phospholipidosis is a manifestation of a specific biochemical property of amiodarone, the inhibition of phospholipid catabolism. At present there is no evidence that it is directly involved in the development of AIPT. Immunopathological processes appear to be associated with AIPT in many patients. It has not been established, however, whether the changes are associated with the cause of lung damage or are the result of AIPT. The development of AIPT in patients without the apparent involvement of such processes would question immunological mechanisms as the only mechanism. Additional research is required to clarify the etiological involvement of immunopathological mechanisms in AIPT.

Either the common mechanism responsible for AIPT has not been discovered, or the pathogenesis of AIPT is multifactorial with possibly different mechanisms, including differences in metabolism of amiodarone, being involved. If preexisting lung disease is a risk factor for AIPT, it is unlikely that a common mechanism for this disorder will be identified. At present, there is unfortunately no unifying theory to explain the development of AIPT in certain individuals, although the involvement of immunopathological mechanisms warrants continued investigation. Studies with animals suggest that oxidant mechanisms may play an important role in AIPT. Further studies related to oxidant mechanisms including the use of antioxidants in animal models and humans to inhibit the development of AIPT appear warranted. It is clear that continued and more innovative research is necessary both to understand the basis for this disorder as well as to develop strategies to reduce its incidence or prevent it altogether.

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