Effect of long-term administration of amiodarone on rat lung and the possible protective role of vitamin E: a histological and immunohistochemical study

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Introduction
Amiodarone is gaining support as a first-line antiarrhythmic drug despite its potentially fatal pulmonary complications involving inflammation and fibrosis.
Objective
This study was undertaken to investigate the effect of long-term amiodarone administration and withdrawal on the histological structure of rat lung. In addition, the possible protective role of vitamin E supplementation was studied.
Materials and methods
This study was carried out on 36 adult male albino rats, which were divided into four groups: group I, which was considered as control; group II (amiodarone-treated group), which received amiodarone orally in a daily dose of 30 mg/kg body weight for 12 weeks; group III (withdrawal group), which received the same dose of amiodarone for 12 weeks and were sacrificed 6 weeks after withdrawal of the drug; and group IV (protected group), which received vitamin E orally in a daily dose of 100 mg/kg body weight simultaneously with amiodarone for 12 weeks. At the time of sacrifice, the lungs were dissected and tissue samples were processed for both light and electron microscopic studies. Immuohistochemical study using anti-CD68 for showing alveolar macrophages was also performed and the number of positively stained cells was morphometrically estimated and statistically analyzed.
Results
Administration of amiodarone alone showed an alteration in the lung architecture in the form of collapsed alveoli with evident proliferation and vacuolation of pneumocytes type II. Thickening of interalveolar septa, cellular infiltration, and collagen deposition associated with an increased number of macrophages as proved by the morphometric study were demonstrated. Intrabronchial cellular debris and vascular congestion were also observed. Coadministration of amiodarone with vitamin E showed a considerable degree of preservation of the pulmonary alveolar architecture; however, a mild improvement in the lung injury was observed in animals after amiodarone withdrawal. The alveoli were lined mainly by vacuolated pneumocytes type II with moderately thickened interalveolar septa.
Conclusion
From this study it could be concluded that prolonged administration of amiodarone in rats can induce severe lung damage. Withdrawal of the drug showed little improvement of this harmful effect but concomitant administration of vitamin E effectively protected the lung tissue. In addition, the increased number of alveolar macrophages associated with amiodarone administration has supported the concept that these cells may play a role in the pathogenesis of lung injury caused by amiodarone.

Keywords:
alveolar macrophages, amiodarone, lung injury, vitamin E

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Introduction

Amiodarone is an iodinated class III antiarrhythmic drug that represents an extremely effective therapy for certain life-threatening cardiac rhythm disturbances [1]. Amiodarone exerts its antiarrythmic property by prolongation of the action potential duration of atrial and ventricular muscles without altering the resting membrane potential [2]. Moreover, the drug slows the heart rate and the atrioventricular nodal conduction through calcium channel and β-adrenergic receptor blockage [3].

Amiodarone is a highly lipid-soluble drug and is stored in high concentration in fat cells and muscles. However, it is associated with a slow onset of action; thus, large loading doses are required before clinical efficacy can be established [4].

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The major metabolite of amiodarone is desethylamiodarone, which is also known to have antiarrythmic properties. The elimination half-life of amiodarone is highly variable and usually long, ranging from 50 to 100 days [5].

Amiodarone is used for long-term management of atrial fibrillation, with prevention of its recurrence. It is also useful for the treatment of supraventricular arrythmias after myocardial infarction [6].

There are numerous side effects associated with amiodarone therapy, including corneal deposits, abnormal liver function tests, thyroid gland dysfunction, bluish discoloration of the skin, bone marrow suppression, coagulopathies, and peripheral neuropathies [7]. However, pulmonary complications represent the most serious adverse reaction limiting the clinical efficacy of this antiarrythmic drug [8]. Lung adverse effects occur in approximately 5% of treated patients. The development of lung complications seems to be associated with older age, long duration of treatment and cumulative dosage, history of cardiothoracic surgery, and probably preexisting lung disease as well as coexisting respiratory infections [9].

Clinical studies have shown an association between amiodarone and a variety of pulmonary complications ranging from subacute necrotizing pneumonitis to pulmonary fibrosis as well as phospholipidosis [10]. These complications have also been reported even with low-dose amiodarone therapy ($\leq$ 200 mg/day) [11].

Several mechanisms have been proposed for the initiation and progression of amiodarone-induced pulmonary toxicity. Considerable evidence suggests that both functional (respiratory and membrane potential) and structural alterations in mitochondria play an initiating role in amiodarone-induced toxicities, including pulmonary toxicity [12].

In addition, a role of oxidative stress in the development of amiodarone-induced pulmonary toxicity has been proposed [13]. It was discovered that amiodarone instillation produces rapid and massive damage to the alveolar–capillary barrier and death to lung airway and parenchymal cells. Meanwhile, amiodarone in solution was found to be capable of generating hydroxyl radicals [14].

Vitamin E plays a leading role in controlling excess oxidative radical formation at cell membranes including mitochondrial membranes [15]. Other physiological functions of vitamin E include restoration of Fas-dependent apoptosis signaling in cancer cells [16], inhibition of superoxide generation in neutrophils [17], and decrease in collagenase expression in fibroblasts [18].

This study was undertaken to investigate the effect of long-term amiodarone administration and withdrawal on the histological structure of rat lung as well as the possible protective role of vitamin E supplementation.

Materials and methods

Animals

Thirty-six adult male albino rats, weighing 150–200 g, were used in this study. The animals were kept in standard housing conditions and were freely supplied with food and water for 1 week before the experiment. The rats were divided into four groups.

Group I (control group)

This group included 12 rats:

Subgroup Ia included four rats that received no treatment and served as control.

Subgroup Ib included four rats that received 3 ml of 0.6% methylcellulose (vehicle of amiodarone) through an orogastric tube daily for 12 weeks. Methylcellulose was obtained from El-Gomhoria, company for chemical and medical trading, Zagazig, Egypt.

Subgroup Ic included four rats that received 1 ml of corn oil/day (vehicle of vitamin E) through an orogastric tube daily for 12 weeks. Half of the animals were killed with group II after a period of 12 weeks, whereas the other half were killed with group III after 18 weeks.

Group II (amiodarone-treated group)

This group included eight rats that received amiodarone (in the form of cordarone 200 mg tablets, Sanofi Pharmaceuticals Company, France) in a daily dose of 30 mg/kg body weight dissolved in 3 ml of 0.6% methylcellulose [19] for 12 weeks by oral gavages [20].

Group III (withdrawal group)

This group included eight rats that received the same dose of amiodarone for 12 weeks and were killed 6 weeks after cessation of amiodarone administration. The reversibility duration of 6 weeks was enough to ensure clearance of the drug from the circulation of rats [21].

Group IV (protected group)

This group included eight rats that received vitamin E (in the form of 400 mg capsules from Pharco Pharmaceuticals Company, Cairo, Egypt) in a daily dose of 100 mg/kg body weight, which was dissolved in 1 ml of corn oil by oral gavages [22], simultaneously with amiodarone for 12 weeks as in group II. The dose of vitamin E was chosen as an effective antioxidant dose in rats [23].

At the end of the experiment, the rats were anesthetized with ether inhalation. The chest was opened and fresh lung specimens were taken and prepared for the following studies.

Histological study

For light microscopic study, specimens were fixed in 10% buffered formalin and 5-μm thick paraffin sections were prepared and stained with hematoxylin and eosin [24] and Mallory's trichrome stains [25].

For electron microscopic study, small pieces of 1 mm^3 of the lung were excised from the diaphragmatic lobe, fixed in 2% gluteraldehyde buffered with 0.1 mol/l phosphate

buffer at pH 7.4 for 2 h at 4°C, and post-fixed in 1% osmium tetroxide. They were then dehydrated with ascending grades of ethanol and placed in propylene oxide for 30 min at room temperature, followed by impregnation in a mixture of propylene oxide and resin (1 : 1) for 1 h and then in a mixture of the previous reagents at 48°C for 1 h. The specimens were embedded in an EM bed-812 resin in BEEM capsules (Pennsylvania) at 60°C for 24 h [26]. Ultrathin sections were cut and double stained with uranyl acetate and lead citrate and were examined with a JEOL transmission electron microscope (JEM 1010, Japan), Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Egypt.

Immunohistochemical study

Immunostaining was performed using an avidin biotin–peroxidase technique for showing alveolar macrophages using CD68 mouse monoclonal antibody (purchased from Novocastra labs, UK, at a dilution of 1 : 20). This antibody has been shown to react selectively with a specific cytoplasmic glycoprotein present in mononuclear phagocytes, microglia, and epidermal langerhans cells [27].

Paraffin sections of the lung were incubated with biotinylated antimouse antibody (diluted 1 : 200) and the avidin biotin-conjugated peroxidase complex (Vector Lab. Inc., USA). The reaction was developed with 0.05% diaminobenzidine (Dakopatts Glostrup, Denmark) as the substrate for peroxidase; finally, the slides were counterstained with Meyer's hematoxylin [28]. The cytoplasmic site of the reaction stained brown whereas the nuclei appeared blue. The specificity of the immune reaction was tested by replacing the primary antiserum with phosphate-buffered saline as a negative control [29].

Morphometric study

The image analyzer computer system Leica Qwin 500, UK in the Histology Department, Faculty of Medicine, Cairo University, was used to count the mean number of alveolar macrophages using the immunostained sections with anti-CD68 monoclonal antibodies. Ten nonoverlapping high-power fields (× 400) from each slide of all animals of each group were used and the positive immunoreactive cells were counted.

Statistical analysis

The data obtained from the image analyzer were expressed as means ± standard deviations. The morphometric results were analyzed using an analysis of variance one-way test. The results were considered statistically significant when the P value < 0.05 and highly significant when the P value < 0.001 [30].

Results

Light microscopic results

Hematoxylin and eosin stain

The lungs of all control subgroups (Ia, Ib, and Ic) showed normal spongy histological structure and architecture of the lung with alveoli, alveolar sacs, thin and thick portions of interalveolar septa, bronchioles, and blood vessels. The alveoli appeared patent with thin interalveolar septa. The lining epithelium of the alveoli was composed of squamous cells (type I pneumocytes) and large cuboidal cells (type II pneumocytes) (Figs 1 and 2).

In the amiodarone-treated group (group II), the lungs showed severe alveolar damage in the form of collapsed alveoli. Markedly thickened interalveolar septa and heavy infiltration of inflammatory cells, mainly lymphocytes associated with thickened wall pulmonary blood vessels, were observed. Congested blood capillaries with extravasation of red blood cells (RBCs) within the alveolar lumen were also detected (Figs 3 and 4a). Most bronchioles showed intrabronchial cellular debris associated with RBCs and alveolar macrophages (Fig. 4b).

Examination of the lung tissue of group III (withdrawal group) showed focal areas of collapsed alveoli with compensatory dilatation of neighboring ones separated by thickened interalveolar septa. Other normal alveoli and moderate interalveolar cellular infiltration were also shown (Fig. 5). Distended wall bronchioles lined by columnar ciliated epithelium and clara cells were observed. Infiltrating inflammatory cells were also detected within the bronchial epithelial lining (Fig. 6).

Concomitant administration of amiodarone with vitamin E (group IV) showed evident reduction of all alveolar changes except for mild thickening of interalveolar septa with mild inflammatory cellular infiltration (Figs 7 and 8).

Mallory's trichrome stain

Lung tissue of all control subgroups showed normal distribution of collagen fibers in the lung parenchyma as fine fibers around the pulmonary blood vessels, and to a lesser extent in the interalveolar septa (Fig. 9).

Lung sections from amiodarone-treated rats (group II) showed an increase in collagen deposition in the thick interalveolar septa as well as around pulmonary bronchioles and blood vessels (Fig. 10), whereas lung tissue of the withdrawal group (group III) showed still-evident increased interstitial collagen (Fig. 11).

Sections of the lung of the protected group (group IV) showed a few collagen fibers in the interalveolar septa, around bronchioles and pulmonary blood vessels (Fig. 12).

Immunohistochemical stain with anti-CD68 showed normal distribution of a few alveolar macrophages in the lung tissue of the control group (Fig. 13).

The amiodarone-treated lung showed markedly increased brown positively stained cells within the interalveolar septa (Fig. 14).

Lung tissue of group III (withdrawal group) showed evident decreased alveolar macrophages in the lung (Fig. 15) but concomitant administration of vitamin E with amiodarone showed a more or less normal distribution of macrophages in the lung parenchyma (Fig. 16).

Ultrastructural results

Group I

Normal architecture was observed in the lungs of all control subgroups. The alveoli appeared patent with thin walls lined by two types of cells: the flattened pneumocyte type I, which is the predominant cell type, and the interspersed large cuboidal pneumocyte type II with rounded euchromatic nuclei and a few short microvilli on their cell surface. Their cytoplasm contained numerous lamellated bodies and mitochondria. The interalveolar septa appeared mostly thin with normal focal areas of thick septum (Figs 17 and 18).

Group II (amiodarone-treated group)

Electron micrographs of amiodarone-treated lung for 12 weeks showed an alteration in the alveolar architecture. The alveoli appeared collapsed and lined predominantly by electron-dense pneumocytes type II. Their cytoplasm depicted atypical vacuolation with degenerative changes of their lamellar bodies leaving irregular empty spaces associated with collagen fiber deposition (Fig. 19).

Alveolar macrophages with large, indented nuclei and many lysosomes were frequently encountered within the alveolar lumen with congested blood capillaries (Fig. 20).

The interalveolar septa were thickened with cellular infiltration of neutrophils and eosinophils. Other septal cells were also observed (Fig. 21).

Group III (withdrawal group)

Electron microscopic examination of the lung tissue after 6 weeks of stopping amiodarone administration showed pneumocytes type II with electron-dense nuclei and vacuolated cytoplasm. Other cells had a normal appearance and retained their characteristic lamellar bodies and other organelles. Alveolar macrophages with long pseudopodia and numerous cytoplasmic lysosomes were also observed. The interalveolar septa showed many collagen fibers (Figs 22 and 23).

Group IV (protected group)

The lung tissue of this group showed a considerable degree of preservation of alveolar architecture. Pneumocytes type II were less frequent. Some of them appeared electron dense with vacuolated lamellar bodies. Others were exfoliated with euchromatic nuclei and characteristic lamellar bodies. The interalveolar septa appeared mildly thickened with cellular infiltration (Fig. 24).

Statistical results

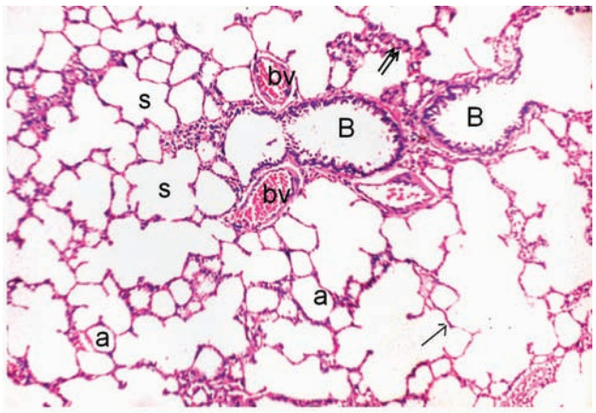
There was a highly significant increase in the number of alveolar macrophages in the amiodarone-treated lung, whereas there was a highly significant decrease in their number in the withdrawal group when both groups were compared with group I (control group). Alveolar macrophages of the protective group showed a nonsignificant increase in their number as compared with the control group (Table 1 and Histogram 1).

Table 1. Mean number of alveolar macrophages/high-power field in the different studied groups

Group	$\bar{X} \pm SD$ (Range)	*P* value
I (control)	2.06 ± 0.92 (0.63–3.85)	
II (amiodarone treated group)	28.98 ± 20.87 (13.48–72.01)	<0.001 HS*
III (withdrawal group)	8.8 ± 5.95 (2.5–15.95)	<0.001 HS*
IV (protected group)	2.75 ± 1.42 (0.63–6.1)	0.21 NS**

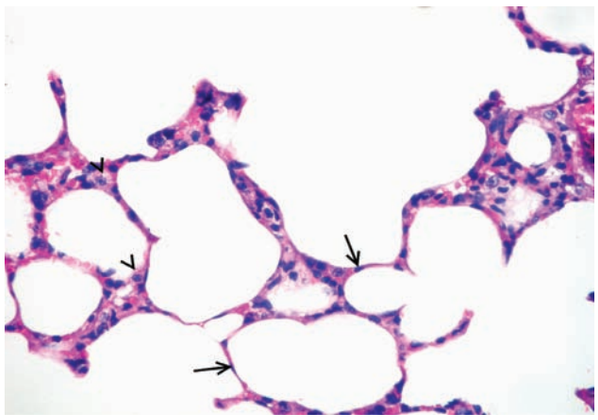
*Indicates highly significant (HS) difference from the control ($P < 0.001$).
**Indicates nonsignificant (NS) difference from the control ($P > 0.05$).

Figure 1.



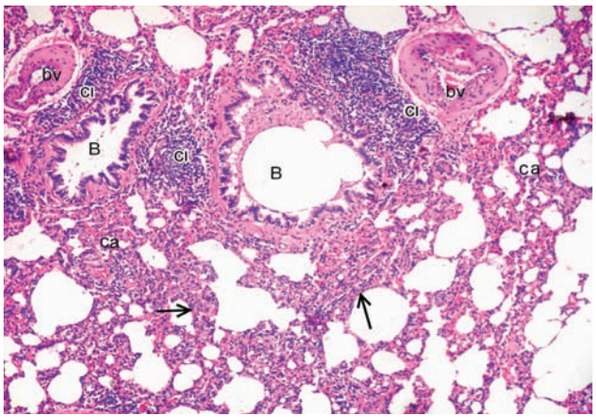
Control rat lung (group I) showing a normal lung architecture with alveoli (a), alveolar sacs (s), thin (↑) and thick (↑↑) portions of interalveolar septa, bronchioles (B), and blood vessels (bv).
H&E × 100.

Figure 2.



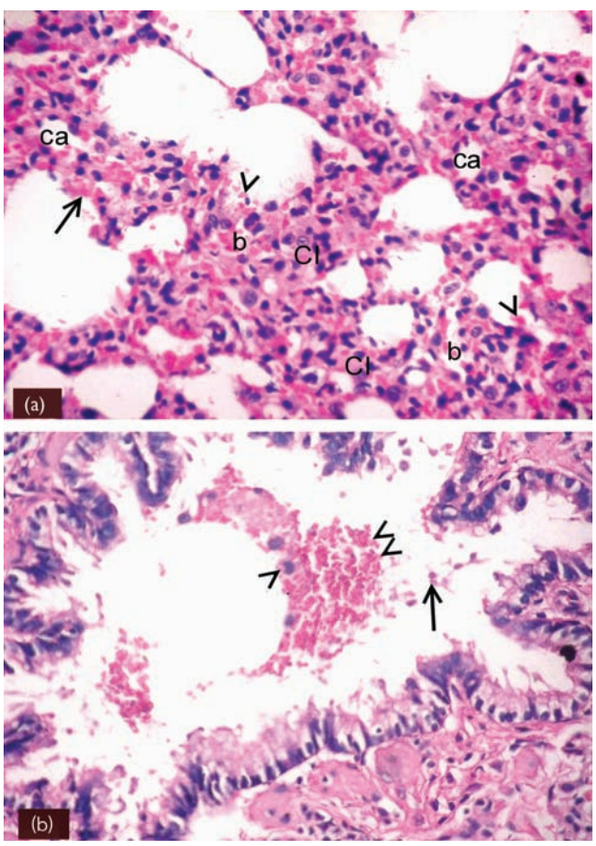
Control rat lung (group I) showing the lining epithelium of alveoli composed of squamous cells (type I pneumocytes) (↑) and large cuboidal cells (pneumocytes type II) (^).
H&E × 400.

Figure 3.



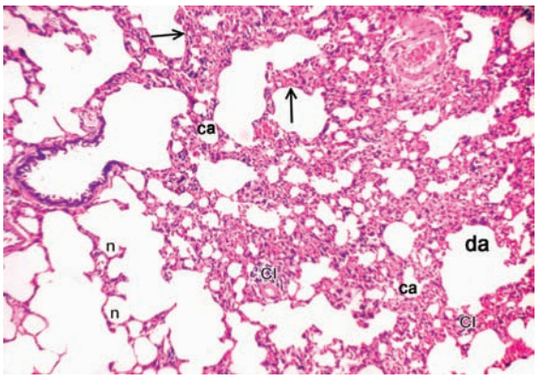
Amiodarone-treated lung (group II) showing marked thickening of interalveolar septa (↑) with interstitial inflammatory cellular infiltration (Cl), which is also seen around bronchioles (B) and thickened wall blood vessels (bv). Severely collapsed alveoli (ca) are also observed.
H&E × 100.

Figure 4.



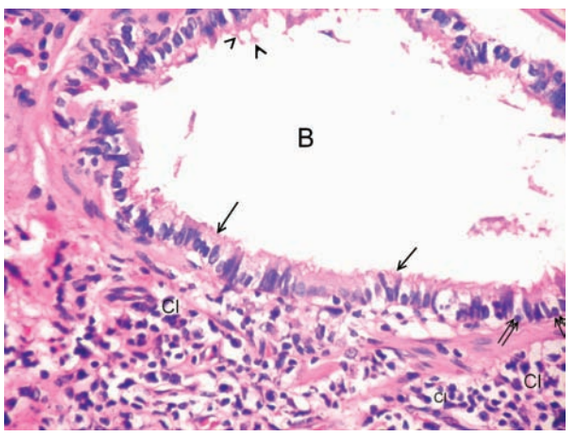
Amiodarone-treated lung (group II) showing (a) collapsed alveoli (ca) separated by thickened interalveolar septa (↑) because of pronounced cellular infiltration (Cl). Congested blood capillaries (b) with extravasation of red blood cells (^) within the alveolar lumen are observed. (b) Intrabronchial cellular debris (↑) associated with RBCs (^^) and alveolar macrophages (^) is also observed.
H&E × 400.

Figure 5.



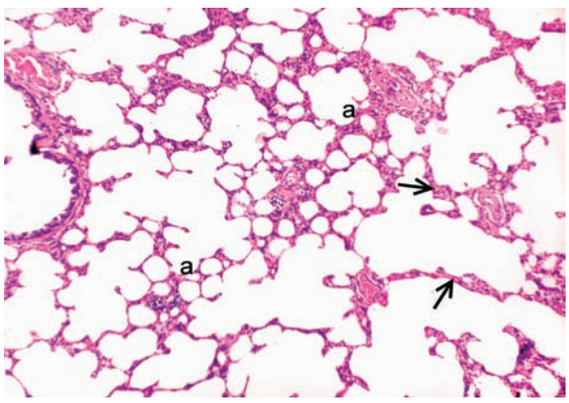
Withdrawal rat lung (group III) showing focal areas of collapsed alveoli (ca) with compensatory dilatation of neighboring ones (da) separated by thickened interalveolar septa (↑). Other normal alveoli (n) and moderate interalveolar cellular infiltration (Cl) are observed.
H&E × 100.

Figure 6.



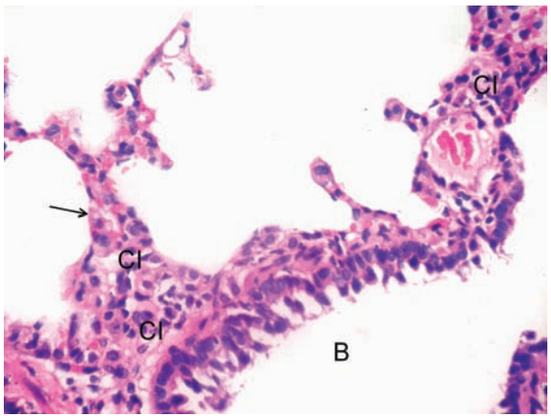
Withdrawal rat lung (group III) showing a distended wall bronchiole (B) lined by columnar ciliated (↑) and nonciliated epithelium (clara cells) (^) with peribronchial cellular infiltration (Cl). Infiltrating cells (↑↑) are detected within the epithelial lining.
H&E × 400.

Figure 7.



Protected rat lung with vitamin E (group IV) showing inflation of most of the alveoli (a) and mildly thickened interalveolar septa (↑).
H&E × 100.

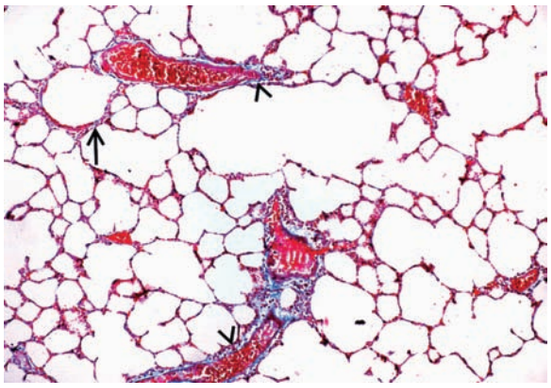
Figure 8.



Protected rat lung with vitamin E (group IV) showing mild inflammatory cellular infiltration (CI) of interalveolar septa (↑) and around a bronchiole (B).

H&E × 400.

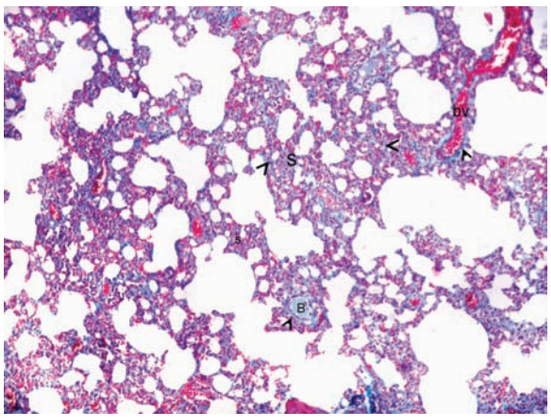
Figure 9.



Control rat lung (group I) showing minimal collagen fibers in the interalveolar septa (↑) and around pulmonary blood vessels (^).

Mallory's trichrome stain × 100.

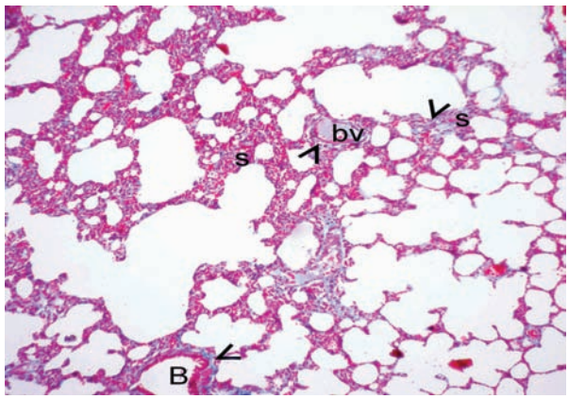
Figure 10.



Amiodarone-treated lung (group II) showing collagen fiber deposition (^) in the thick interalveolar septa (s) as well as around pulmonary bronchioles (B) and blood vessels (bv).

Mallory's trichrome stain × 100.

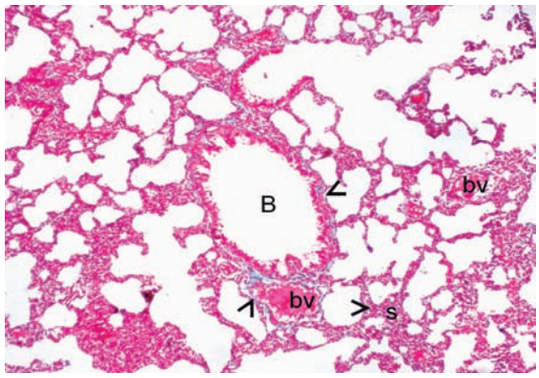
Figure 11.



Withdrawal rat lung (group III) showing a mild increase in the deposition of collagenous fibers (^) around a blood vessel (bv), a bronchiole (B), and in the mildly thickened interalveolar septa (s).

Mallory's trichrome × 100.

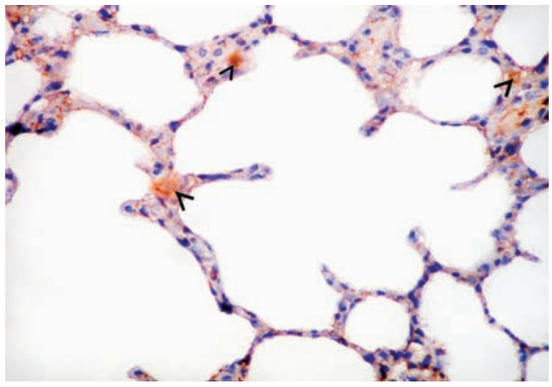
Figure 12.



Protected rat lung with vitamin E (group IV) showing a few collagen fibers (^) in the interalveolar septa (s), around a bronchiole (B), and pulmonary blood vessels (bv).

Mallory's trichrome × 100.

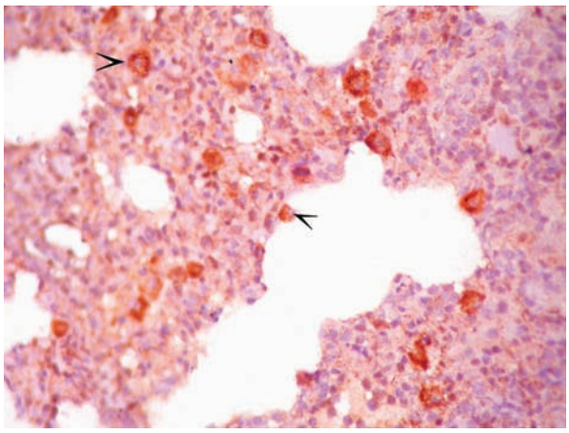
Figure 13.



Control rat lung (group I) showing a few brown positively staining cells of alveolar macrophages (^).

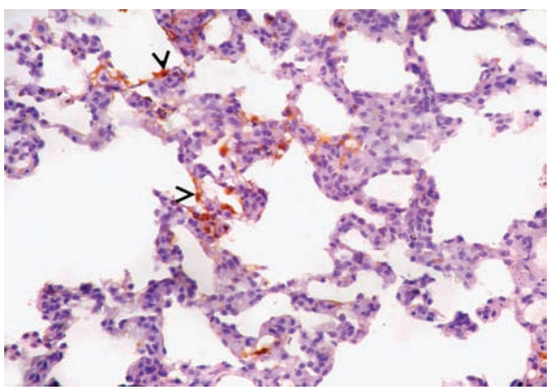
Anti-CD68 × 400.

Figure 14.



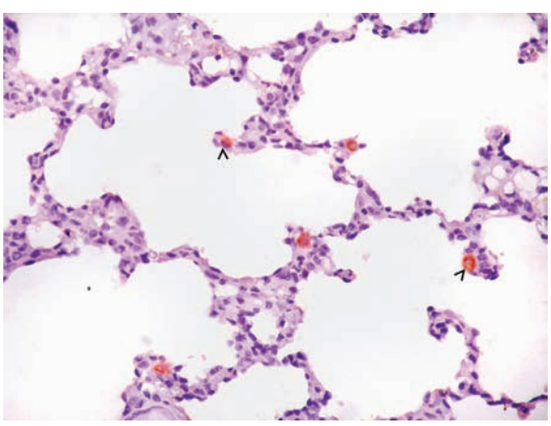
Amiodarone-treated lung (group II) showing many brown positively staining cells (^) for alveolar macrophages in the interalveolar septa.
Anti-CD68 × 400.

Figure 15.



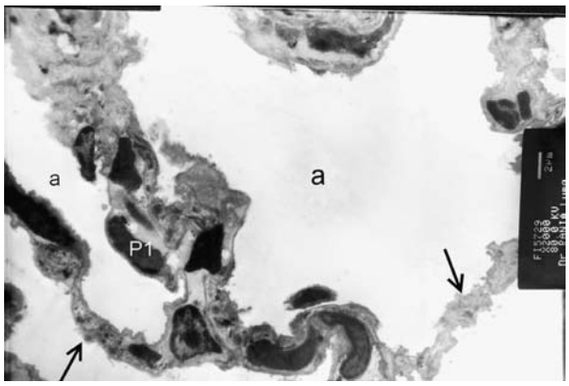
Withdrawal rat lung (group III) showing a mild decrease of brown positive staining of alveolar macrophages (^) compared with the control group.
Anti-CD68 × 400.

Figure 16.



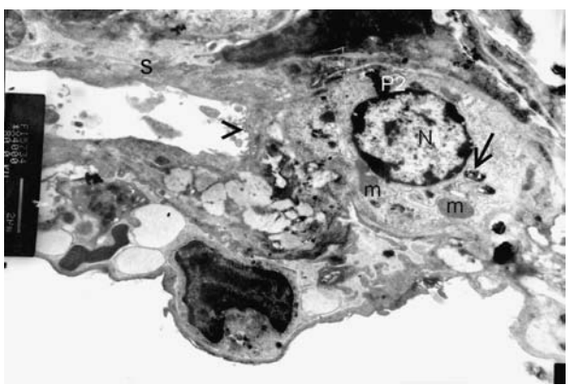
Protected rat lung with vitamin E (group IV) showing normal brown positive staining for alveolar macrophages (^).
Anti-CD68 × 400.

Figure 17.



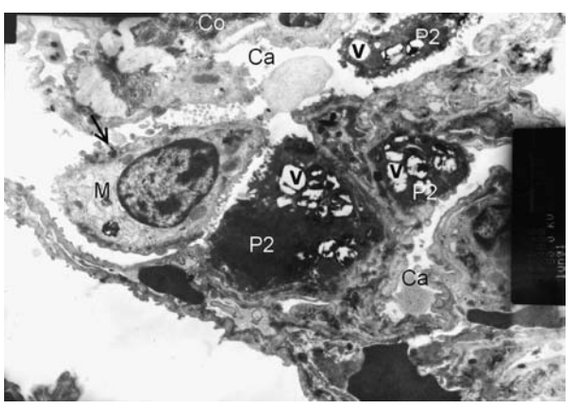
Control rat lung (group I) showing alveoli (a) lined by pneumocytes type I (P1) separated by thin interalveolar septa (↑).
Transmission electron microscopy × 4500.

Figure 18.



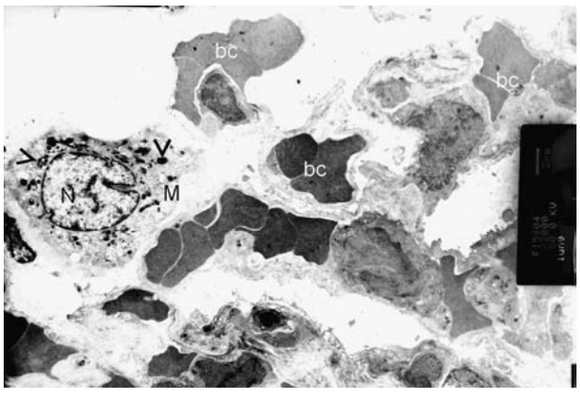
Control rat lung (group I) showing a thick portion of the interalveolar septa (S) with pneumocytes type II (P2) having large rounded euchromatic nucleus (N) and short microvilli on the cell surface (^). Its cytoplasm shows lamellar bodies (↑) and mitochondria (m).
Transmission electron microscopy × 9000.

Figure 19.



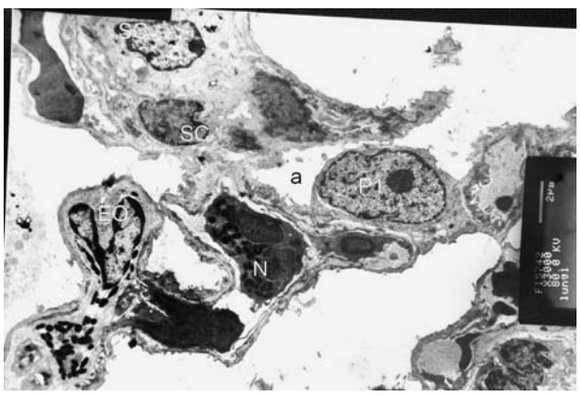
Amiodarone-treated lung (group II) showing collapsed alveoli (ca) lined by many electron-dense pneumocytes type II (P2) with degenerative changes of their lamellar bodies leaving irregular empty vacuoles (v). An intra-alveolar macrophage (M) with pseudopodia (↑) and interstitial collagen fibers (Co) are also observed.
Transmission electron microscopy × 9000.

Figure 20.



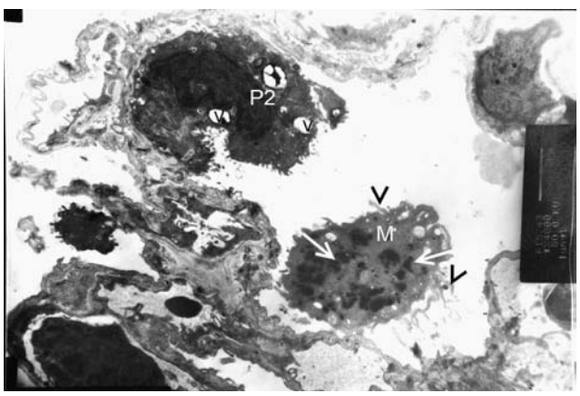
Amiodarone-treated lung (group II) showing an alveolar macrophage (M) with large indented nucleus (N) and many cytoplasmic lysosomes (^). Congested blood capillaries (bc) are also observed.
Transmission electron microscopy × 4000.

Figure 21.



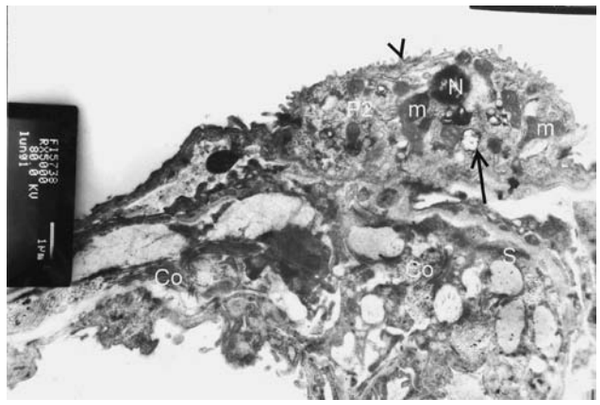
Amiodarone-treated lung (group II) showing a neutrophil (N) and an eosinophil (EO) infiltrating the interalveolar septa. A pneumocyte type I (P1) is observed lining the wall of an alveolus (a). Notice the presence of interstitial septal cells (SC).
Transmission electron microscopy × 9000.

Figure 22.



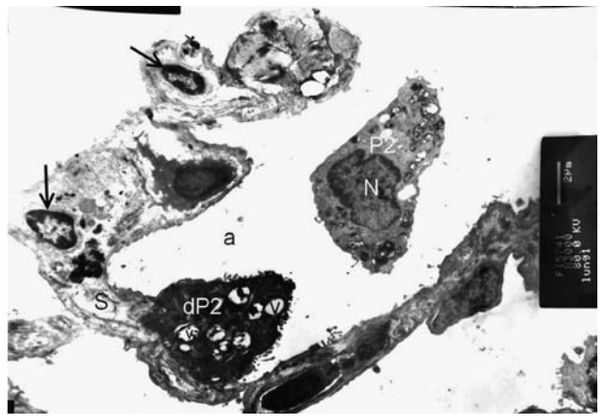
Withdrawal rat lung (group III) showing a pneumocyte type II (P2) with electron-dense nucleus and vacuolated (v) cytoplasm. An alveolar macrophage (M) with long pseudopodia (^) and numerous cytoplasmic lysosomes (↑) is also observed.
Transmission electron microscopy × 9000.

Figure 23.



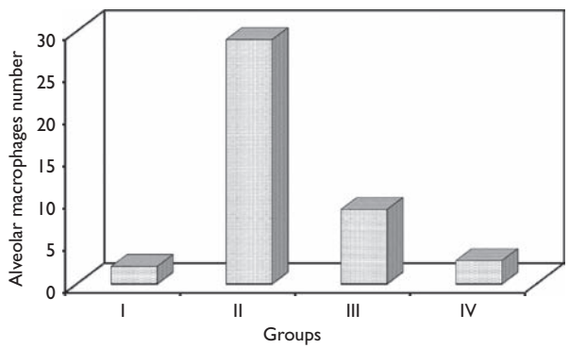
Withdrawal rat lung (group III) showing a pneumocyte type II (P2) with small, electron-dense nucleus (N) recognized by its microvillous border (^), lamellar bodies (↑), and mitochondria (m). Collagen fiber deposition (Co) within the interalveolar septa (S) is also observed.
Transmission electron microscopy × 10000.

Figure 24.



Protected rat lung with vitamin E (group IV) showing mild thickening of interalveolar septa (S) associated with cellular infiltration (↑). An electron-dense pneumocyte type II (dP2) with vacuolated lamellar bodies (v) is observed lining the wall of an alveolus (a). Another exfoliated pneumocyte type II (P2) with euchromatic nucleus (N) and characteristic lamellar bodies (^) is also detected.
Transmission electron microscopy × 6500.

Histogram 1.



The mean number of alveolar macrophages in the different studied groups.

Discussion

Amiodarone is a potent antiarrhythmic agent effective in the prophylaxis and treatment of many forms of life-threatening cardiac arrythmias and consequently in the prevention of sudden cardiac death [31,32]; however, its use is often associated with serious pulmonary complications, the most common of which is chronic interstitial pneumonitis with organizing pneumonia and pulmonary fibrosis [33].

In this study, examination of the lungs of the amiodarone-treated group showed severe alveolar damage in the form of collapsed alveoli, heavy infiltration of thickened interalveolar septa with inflammatory cells, mainly lymphocytes associated with congested blood capillaries, and extravasation of RBCs within the alveolar lumen. Ultrastructurally, proliferation and atypical vacuolation of pneumocytes type II with degenerative changes in their lamellar bodies leaving irregular empty spaces and few lipid-containing vacuoles were also detected.

The alveolar collapse observed in rats treated with amiodarone might be attributed to the imbalance between production and degradation of the surfactant. Excessive production of surfactant by hyperplastic pneumocytes type II seemed to exceed the ability of alveolar macrophages to degrade it [34].

Several mechanisms of amiodarone adverse pulmonary effects have been proposed, including direct cellular damage, induction of phospholipidosis, and immune-mediated mechanisms such as the activation of natural killer cell activity [35].

It was reported that amiodarone induces phospholipidosis in humans and animals because of the inhibition of lysosomal phospholipases resulting in an abnormal degradation of phospholipids promoting its intracytoplasmic accumulation and permitting phagocytic cells to accumulate large quantities of lipids leading to the appearance of vacuolated pneumocytes type II and foamy macrophages [36].

Proliferation of pneumocytes type II to form predominant lining cells of the alveoli was in accordance with the previous experimental studies that suggested that pneumocytes type II might constitute the reserve epithelial cells of the alveoli, and its proliferation and hyperplasia were regarded as a manifestation of pneumocyte type I repair and reflected its underlying injury [37].

This study showed thickening of interalveolar septa, which could be explained by the increased interstitial collagen fiber deposition and marked cellular infiltration with lymphocytes, neutrophils, eosinophils, and macrophages. Moreover, a significant increase in macrophage number was proved by the morphometric study. This finding was previously reported by other investigators [38] who stated that alveolar macrophages could release many mediators such as tumor necrotizing factor, which augment the inflammatory response of airways and alveoli. In addition, alveolar macrophages release a chemotactic substance specific for neutrophils, which in turn release proteases and toxic oxygen-free radicals that increase the destruction of tissue and maintain alveolitis [34].

In contrast, alveolar macrophage number was less in the withdrawal group when compared with their number in the control group but concomitant administration of vitamin E with amiodarone showed a more or less normal distribution of macrophages in the lung parenchyma. These results might be because of the inhibition of the production of monocyte chemoattractant protein-1 by vitamin E [39].

Amiodarone pulmonary toxicity can progress to fibrosis. Pulmonary fibrosis is a chronic and incurable respiratory disease with abnormal deposition of collagen following tissue damage [40]. It is believed that injury to the epithelium and basement membranes is a requisite step in the etiology of pulmonary fibrosis [41], after which several cell types, including inflammatory and immune cells as well as fibroblasts, migrate to and/or proliferate in areas of injury and release numerous cytokines that lead to further cell recruitment, inflammation, and eventual matrix remodeling. This culminates in an overproduction of collagen and other matrix components that are characteristic of fibrosis [42].

In contrast, previous researchers [43] have postulated that intratracheal administration of amiodarone hydrochloride is the only route of amiodarone administration that can cause pulmonary toxicity, including fibrosis in experimental animals. The proliferative rate of various pulmonary cells in normal lung is low; this includes fibroblasts that must be under control to prevent the development of fibrotic lung. Most researchers [44] have focused on the role of macrophage-derived factor in the control of fibroblast proliferation and collagen synthesis. Other studies added that pneumocytes type II have been shown to secrete prostaglandin E2, which can act to suppress fibroblast growth [45,46].

Vascular congestion and cellular infiltration of the lung tissue observed in this study could be referred to changes of the vascular integrity of the lung vessels causing disruption of the endothelial barrier and increased capillary permeability evoking an inflammatory response through activation of oxidative stress-sensitive signaling pathways [47]. In addition, the intrabronchial cellular debris observed in most bronchioles was attributed to the direct toxic effect of amiodarone on mucosa-lining bronchioles [44].

The incomplete reversibility of the histological changes of lung tissue after amiodarone withdrawal might be explained by the long half-life of the drug and its tendency to accumulate in the lung [48].

Concomitant administration of vitamin E with amiodarone for 12 weeks (group IV) showed a considerable protection of the lung tissue. The lung architecture was considerably preserved; the alveoli were distended and the interalveolar septa were mildly thickened because of focal increase in collagen and mild

cellular infiltration. Ultrastructurally, pneumocytes type II were less frequent. Some of them appeared electron dense with vacuolated lamellar bodies. Others regained their normal appearance with euchromatic nuclei and characteristic lamellar bodies.

These changes were previously described by other researchers [49] who reported that dietary vitamin E supplementation has prevented alveolar damage and reduced the extent of pulmonary collagen deposition caused by intratracheal amiodarone. The protection offered by vitamin E *in vitro* and *in vivo* may be the result of several effects, including decreased cellular amiodarone accumulation, membrane stabilization, altered profibrotic gene expression, and free radical scavenging [50].

Moreover, it had been suggested that the positive effect of vitamin E in pulmonary fibrosis can be attributed to the inhibition of release of cytokines released from macrophages, such as transforming growth factor-β_1, which is most important in extracellular matrix remodeling [51,52].

Vitamin E has been reported to decrease amiodarone-induced cytotoxicity in cultured pulmonary and nonpulmonary cells, whereas other antioxidant treatments were ineffective [53].

In a hamster model of amiodarone-induced pulmonary toxicity, dietary vitamin E supplementation substantially reduced the extent of pulmonary collagen deposition and histological damage after intratracheal amiodarone administration [54]. Furthermore, vitamin E has prevented cellular infiltration and thickening of the interstitial spaces [55].

Conclusion

From the previous results, it could be concluded that prolonged administration of amiodarone in rats can induce severe lung damage. Withdrawal of the drug showed little improvement of this harmful effect but concomitant administration of vitamin E effectively protected lung tissue. In addition, the increased number of alveolar macrophages associated with amiodarone administration has supported the concept that these cells may play a role in the pathogenesis of lung injury caused by amiodarone.

Therefore, patients treated with amiodarone must be carefully selected and subjected to periodic evaluation with chest radiograph and pulmonary function tests to detect early pulmonary changes. It is also advised to alleviate toxicity and prolong the usefulness of amiodarone by concomitant administration of vitamin E, which effectively protected lung tissue against amiodarone toxicity.

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الملخص العربي

تأثيرالاستخدام الطويل لعقار الأميوداروون على رئة الجرذ والدور الوقائى المحتمل لفيتامين (هـ) (دراسة هستولوجيه وهستوكيميائيه مناعية)

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يعتبر عقار الأميودارن الخط العلاجى الأول فى كثير من أمراض القلب بالرغم من مضاعفاته الكثيرة و بخاصة تأثيره على الرئة ولذلك كان الهدف من هذا البحث هو التعرف علىالتغيرات الهستولوجية المصاحبة لعقار الأميودون علىالنسيج الرئوى مع دراسه التأثير الوقائى المحتمل لفتامين (هـ).

تم فى هذا البحث استخدام 36 من ذكور الجرذان البيضاء البالغة تم تقسيمهم الى اربعة مجموعات المجموعة الأولى مجموعة ضابطة والمجموعة الثانية لدراسة تأثير عقارالأميوداروون بجرعة 30مجم/ كج عن طريق الفم لمدة12 أسبوع أما المجموعة الثالثة فبعد حصولها على الأميوداروون كالمجموعة الثانيةأوقف العلاج لمدة ستة أسابيع أخرى لمتابعة تأثيرسحب العقار و المجموعة الرابعة لدراسة تأثير إستخدام فيتامين (هـ) متزامناً مع الأميوداروون بجرعة تبلغ100مجم/ كجم عن طريق الفم لمدة12 أسبوع. و قد تم اخذ عينات من الرئه
و تحضيرها للفحص بالمجهرين الضوئي والإلكتروني مع اجراء دراسة هستوكيميائيه مناعية على الخلايا الملتهمه بالرئة.

وقد أوضحت النتائج ان عقار الاميوداروون قد أدى الى حدوث العديد من التغيرات الهستولوجية فى التركيب النسيجي للرئه منها زيادة سمك الحاجز بين الحويصلات الهوائية وتورم فى النسيج البينى وذلك نتيجة زيادة الخلايا الالتهابية ووجود كرات دموية حمراء من الشعيرات الدمويه المحتقنة الى المسافات البينيه مع تزايد ألياف الكولاجين خصوصا حول الشعيبات الهوائية والاوعية الدمويه وكذلك أدى الاميوداروون الى زيادة الخلايا الهوائية من النوع الثانى مع نقص الحبيبات ذات التركيبات الحلزونيه بها ووجود العديد من الفجوات، كما أثبت الفحص الهستوكيميائى المناعى زيادة فى عدد خلايا الرئتين الملتهمه الكبيرة بدرجة ذات دلالة احصائية عالية وذلك مقارنةبالمجموعات الأخرى كما وجد أن توقف العلاج لمدة 6 أسابيع لم يحدث الا تحسنا طفيفا في التركيب النسيجى للرئة اما بعد استخدام فيتامين (هـ) فقد حدث تحسن كبيرفى النسيج الرئوى واحتوى على شعيبات و حويصلات هوائية سليمة نسبيا .

ويمكن أن نستخلص من هذا البحث أن عقار الأميوداروون قد احدث تغيرات هستولوجية خطيرة فى الرئة و أن لفتيامين (هـ) تأثيرا وقائيا على تلك الآثار لذلك يوصى باستخدامه للتقليل من الآثار الجانبية للاميوداروون.