Effects of metabolites and analogs of amiodarone on alveolar macrophages: structure-activity relationship

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Quaglino, Daniela, Huy Riem Ha, Elena Duner, Daniela Bruttomesso, Laurent Bigler, Ferenc Follath, Giuseppe Realdi, Andrea Pettenazzo, and Aldo Baritussio. Effects of metabolites and analogs of amiodarone on alveolar macrophages: structure-activity relationship. Am J Physiol Lung Cell Mol Physiol 287: L438–L447, 2004. First published April 9, 2004; 10.1152/ajplung.00434.2003.—Amiodarone, an antiarrhythmic drug toxic toward the lung, is metabolized by the liver to diethylaminoethoxy metabolites. These metabolites can also be transformed by dealkylation to mono- and dibutyl derivatives of the diethylaminoethoxy group, which are toxic toward the lung. We exposed alveolar macrophages to these metabolites and analogs of amiodarone and examined their effect on the cell surface and endocytic pathway. The substances studied included MDEA, DDEA, and B2-O-EtOH, analogs with different modifications of the diethylaminoethoxy group, fragments of the amiodarone molecule, and the antiarrrhythmic agents dronedarone (SR-33589) and KB-130015. We found the following: 1) MDEA, DDEA, and B2-O-EtOH rank in order of decreasing toxicity toward alveolar macrophages, indicating that dealkylation and reamination of the diethylaminoethoxy group represent important mechanisms of detoxication; 2) dronedarone has greater, and KB-130015 has smaller, toxicity than amiodarone toward alveolar macrophages; and 3) the benzofuran moiety, which is toxic to liver cells, is not directly toxic toward alveolar macrophages.

AMIODARONE (Fig. 1) is a class III antiarrhythmic agent used extensively to treat ventricular and supraventricular arrhythmias and to prevent atrial fibrillation in patients undergoing cardiac surgery (16). Despite recognized efficacy and wide use, amiodarone poses problems to clinicians because of its peculiar pharmacokinetics and toxicity. After absorption, amiodarone leaves the serum compartment with a half-life of 10–20 h and accumulates in peripheral tissues to very high levels, so that when the dosing rate is reduced or halted, the accumulated amiodarone moves back into serum, resulting in a long terminal elimination half-life (18). The tissues where amiodarone accumulates most include adipose tissue, liver, skeletal muscle, lung, pancreas, thyroid gland, kidney, heart, skin, adrenal glands, testis, and lymph nodes. In the liver and other tissues, amiodarone undergoes dealkylation to mono-N-desethylamiodarone (MDEA), a derivative with antiarrhythmic activity, propensity to accumulate into tissues, and a half-life similar to those of the parent drug but with greater toxicity (18). MDEA can be further transformed by dealkylation to di-N-desethylamiodarone (DDEA), by deamination to amiodarone-EtOH (B2-O-EtOH), and by hydroxylation to n-3’-hydroxybutyl-N-desethylamiodarone, whose biological effects are presently unknown (unpublished observations). Further degradation of these compounds probably occurs, since several studies have reported an increased excretion of free iodine during amiodarone therapy (18). Several P450 cytochromes, such as 3A4, 2D6, 1A1, and 1A2, are involved in amiodarone degradation (17).

Amiodarone metabolites MDEA, DDEA, and B2-O-EtOH are formed in vivo through sequential modifications of the diethylaminoethoxy group, but the relevance of these changes to lung cells is unclear. In this paper, to clarify this point, we exposed rabbit alveolar macrophages to analogs of amiodarone with different modifications of the diethylaminoethoxy group (Fig. 2) and then searched for biochemical signs of cell damage, formation of vacuoles and inclusion bodies, and interference with the degradation of surfactant protein A, used as a tracer of the endocytic pathway. The substances studied included MDEA, DDEA, and B2-O-EtOH, analogous with different modifications of the diethylaminoethoxy group, fragments of the amiodarone molecule, and the antiarrrhythmic agents dronedarone (SR-33589) and KB-130015. We found the following: 1) MDEA, DDEA, and B2-O-EtOH rank in order of decreasing toxicity toward alveolar macrophages, indicating that dealkylation and reamination of the diethylaminoethoxy group represent important mechanisms of detoxication; 2) dronedarone has greater, and KB-130015 has smaller, toxicity than amiodarone toward alveolar macrophages; and 3) the benzofuran moiety, which is toxic to liver cells, is not directly toxic toward alveolar macrophages.

Dronedarone; KB-130015

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MATERIALS AND METHODS

The experiments were approved by the local committee on the handling of laboratory animals.

Materials. All reagents were of analytical grade. Sodium 125I was from Amersham Pharmacia Biotech (Little Chalfont, UK).

Drugs. Amiodarone-hydrochloride was from Sigma. MDEA-hydrochloride [2-n-Butyl-3-(3,5-diido-4-ethylaminoethoxybenzoyl)-benzo-furan] was a gift from Sanofi (Munchenstein, Switzerland). KB-130015...
was a gift from Dr. Bo Carlsson (Caro Bio, Novum, Huddinge, Sweden). Dronedarone (SR-33589) was a gift from Sano-fi-Synthelabo (Chilly-Mazarin Cedex, France). MeAMI (2-butyl-benzofuran-3-yl)-4-[2-(dimethylamino-ethoxy)-3,5-diiodophenyl]-methanone, DDEA (2-butyl-benzofuran-3-yl)-4-[2-(aminoethoxy)-3,5-diiodophenyl]-methanone hydrochloride, B2-O-AcOH (2-n-butyl-3-(3,5-diido-4-carboxymethoxysbenzyl)benzofuran), B2-O-EtOH, B2-O-Et (2-butyl-benzofuran-3-yl)-(4-ethoxy-3,5-diiodophenyl-methanone), B2 [2-butyl-benzofuran-3-yl)-(4-hydroxy-3,5-diiodophenyl)-methanone], D2 [4-(2-diethylaminoethoxy)-3,5-diiodobenzoic acid], and DIB-O-A (4-carboxymethoxy-3,5-diiodobenzoic acid) were synthesized in the Cardiovascular Therapy Research Laboratory of the Zurich University Hospital (13). The chemical structure of newly synthesized compounds was confirmed by UV, liquid chromatography-electrospray ionization mass spectroscopy/mass spectroscopy, and nuclear magnetic resonance (1 H and 13 C) spectroscopies. Purity was >98%. A detailed description of the synthesis and analytical data supporting the chemical structure of compounds used here will be published elsewhere. Drugs were prepared as 50 mM stock solutions in DMSO and stored at 26°C. Drugs were added to cells in 1/1000 ml of DMSO/ml. No endotoxin could be detected into these solutions using the Limulus amebocyte lysate (Bio-Whittaker, Cambrex, Walkersville, MD; lowest limit of detection 0.06 endotoxin units/ml).

Measurement of drug lipophilicity. Lipophilicity was estimated as described by Zamora et al. (22) with minor modifications. Briefly, HPLC-grade 1-octanol (Sigma) was presaturated with 0.1 M PBS, pH 7.2, and conversely, PBS was presaturated with 1-octanol. Drugs were then dissolved in PBS at a final concentration of 10⁻⁴ M, an equal volume of 1-octanol was added, and the tubes were continuously inverted for 15 min. Drug concentrations in the aqueous and octanol phases were assessed by measuring the absorbance at the most convenient wavelength, as determined from the UV spectra. The partition coefficient D was calculated by dividing the absorbance in 1-octanol by the absorbance in PBS. Log₁₀ D was used as a measure of lipophilicity (Fig. 2).

Cells. Alveolar macrophages, obtained by washing the airways of adult rabbits with 145 mM NaCl, 5 mM KCl, 2.5 mM Na₂HPO₄, 2 mM HEPES, 6 mM glucose, and 0.2 mM EGTA, pH 7.4, were washed two times with Ringer buffer (145 mM NaCl, 5 mM KCl, 2 mM Na₂HPO₄, 1 mM MgCl₂, 2 mM HEPES, 10 mM glucose, pH 7.4), suspended in Ringer buffer plus 1 mg/ml of BSA (RBA), and used immediately. The cells were >90% macrophages (May-Grunwald-Giemsa staining) and 95 ± 1% viable (means ± SE, n = 47) as determined by trypan blue exclusion.

Effect of drugs on trypan blue exclusion and on release of lactate dehydrogenase by alveolar macrophages. Macrophages (10⁶ cells in 1 ml of RBA) were incubated for 1 h at 37°C with amiodarone or amiodarone analogs, added in 1 μl of DMSO. At the end, a small aliquot was used to study trypan blue exclusion, whereas the remaining cells were sedi-

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<th>Compound</th>
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Fig. 2. Amiodarone analogs. Log D = Log₁₀ (absorbance of 1-octanol phase/absorbance of water phase), average of 3 determinations. MDEA, mono-N-desethylamiodarone; DDEA, di-N-desethylamiodarone. See MATERIALS AND METHODS for description of abbreviated compounds.
mented at 3,000 rpm, and lactate dehydrogenase (LDH) released in 50 μl of the supernatant was measured with a commercial kit (CytoTox 96, Promega). LDH released is presented as % of the LDH liberated from the same cells in the presence of 0.9% Triton X-100 and is compared with LDH released from cells exposed to plain DMSO (control macrophages). Under the present conditions, control macrophages released 2.4 ± 0.7% of total LDH per hour (means ± SE), n = 6.

Effect of amiodarone and amiodarone analogs on alveolar macrophage morphology. Macrophages in RBA plus 50 units/ml of penicillin and 50 μg/ml of streptomycin were allowed to adhere for 2 h to six-well Falcon plates (Becton Dickinson Labware Europe, Meylan, France; 2 × 105 cells/plate) and then were cultured for 24 h at 37°C, 5% CO2, in the presence of DMSO (1 μM/ml control cells), 10 μM amiodarone, or 10 μM amiodarone analogs. At the end, adhering cells were washed with Tyrode buffer, pH 7.3, scraped, and centrifuged at 10,000 g. The resulting pellets were fixed overnight with 2.5% glutaraldehyde in Tyrode buffer, postfixed for 2 h in 1% osmium tetroxide, dehydrated, and embedded in Spurr resin. Semithin sections obtained through the whole thickness of the pellets were stained with toluidine blue and observed with a Zeiss Axiophot light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Jeol 1200 EXGII electron microscope.

Morphometry was performed on 10 electron micrographs of each experimental condition randomly taken at ×4,000 magnification and then photographically enlarged to ×10,000 magnification to appreciate the details of at least 2–3 cells/micrograph. A total number of 25 cells in each experimental condition were counted. By means of a ruler inserted within an optical magnifier, we measured the surface area covered by the whole cell and by vacuoles or cytoplasmic inclusions (i.e., electron-dense structures containing amorphous and/or multilamellar membranes, not surrounded by any type of membrane). All organelles that were detectable in the section of each of the randomly selected cells were analyzed. Results are expressed as % of the surface area covered by different organelles in relation to the surface area covered by the whole cytoplasm.

Preparation and labeling of SP-A. SP-A was isolated from the surfactant obtained from a patient with alveolar proteinosis and labeled with sodium 125I as described (4). 125I-SP-A had a specific activity of 400–600 cpm/ng, migrated as expected during polyacrylamide gel electrophoresis, and was >99% precipitable with 20% cold TCA. 125I-SP-A was stored at 4°C and used within a month.

Effect of amiodarone and amiodarone analogs on the degradation of 125I-SP-A by alveolar macrophages. Alveolar macrophages (106 cells in 1 ml of RBA) were incubated for 1 h at 37°C in the presence of different drugs added in 1 μl of DMSO (final drug concentration 0–50 μM). One microgram of 125I-SP-A was then added, and the incubation was continued for one further hour. At the end, the radioactivity soluble in 20% cold TCA was measured in medium plus cells. Degradation of SP-A is presented as % of the degradation of SP-A measured in control cells. Under the present conditions, control cells degraded 46 ± 7 ng of SP-A/106 cells/hour (means ± SE, n = 30).

Effect of amiodarone, dronedarone, and KB-130015 on the clearance of 125I-SP-A administered into trachea. Sedimentable surfactant (fraction B of Ref. 3) freshly obtained from 3-day-old rabbits was suspended in saline, mixed with 125I-SP-A, and then combined with amiodarone, dronedarone, KB-130015, or plain DMSO to make four different mixtures. Two hundred microliters of each mixture contained: 1 μg of 125I-SP-A, 5% of the alveolar pool of phospholipids normally found in 3-day-old rabbits, 250 mmol of one of the drugs in 5 μl of DMSO, or 5 μl of plain DMSO (controls). Surfactant was added to the mixtures to prevent nonspecific binding of SP-A to vials or tubing.

Two hundred microliters of the mixtures were instilled to 3-day-old rabbits (10 rabbits per mixture) by puncturing the trachea after exposing it to local anesthesia. The animals were killed after 3 h with an excess of pentobarbital sodium in the peritoneum, and the airways were lavaged four times with 4–5 ml of 0.9% normal saline. The lavage fluid was used to count cells, to estimate cell viability by trypan blue exclusion, and to measure total and TCA-soluble radioactivity. An aliquot of it was centrifuged for 5 min at 500 g, and the supernatant was used to measure proteins and LDH. The washed lungs were homogenized in cold saline, and the radioactivity of the homogenate, total and TCA-soluble, was measured. The radioactivity recovered from lavage fluid and homogenate was expressed as % of the dose instilled.

The animals were killed 3 h after instillation because, after this time interval, administered SP-A is evenly distributed between alveoli and parenchyma and the lung retains enough radioactivity for counting (4). The dose of 250 nmol was chosen because we previously found that 250 nmol of amiodarone instilled into trachea interfere with the clearance of SP-A without clear damage to the lungs (4).

Statistical analysis. Data are expressed as means ± SE. Differences between groups were analyzed by ANOVA using the Student-Newman-Keuls test as the post hoc test for data normally distributed and Dunn’s test for data not normally distributed. The level of significance accepted was 5%.
RESULTS

Signs of cell damage. Amiodarone had no effect on trypan blue exclusion up to a concentration of 20 μM. MDEA and DDEA were more powerful than amiodarone, exhibiting a significant inhibition at a concentration of 20 μM (Fig. 3). Dronedarone and MeAMI had an effect similar to that of amiodarone. B2, B2-O-Et, B2-O-EtOH, B2-O-AcOH, and KB-130015 had no effect on trypan blue exclusion up to a concentration of 50 μM (Fig. 3). Analogs lacking the benzofuran group, like the hydrophilic compounds D2 and DIB-O-A, had no effect on trypan blue exclusion up to a concentration of 50 μM (not shown).

Thus, considering amiodarone metabolites, it appears that MDEA and DDEA are more toxic than amiodarone, whereas B2-O-EtOH has no effect on trypan blue exclusion. Considering all substances studied, it appears that only analogs with an amine function and ranking at the top of the hydrophobicity scale (MDEA, DDEA, MeAMI, and dronedarone) interfere with trypan blue exclusion (Figs. 2 and 3). The finding that the hydrophilic analog D2, which lacks the benzofuran moiety but retains a complete diethylaminoethoxy group, had no effect on trypan blue exclusion, suggests that a certain degree of hydrophobicity is required to affect trypan blue exclusion.

Incubation with 1–50 μM amiodarone had a modest effect on the release of LDH by alveolar macrophages, whereas MDEA, DDEA, and dronedarone induced a dramatic increase (Fig. 4). MeAMI also increased the release of LDH, but the change was not significant (Fig. 4). B2, B2-O-Et, B2-O-EtOH, B2-O-AcOH, and KB-130015 did not increase the release of LDH by alveolar macrophages (Fig. 4). These results reinforce the concept that 1) amiodarone metabolism generates derivatives with diverse toxicity toward alveolar macrophages, some being more (MDEA and DDEA) and some being less (B2-O-EtOH) toxic than the parent compound and 2) dronedarone is more, and KB-130015 is less, toxic than amiodarone toward alveolar macrophages. Furthermore, because the uptake of trypan blue and the release of LDH are both due to damage to the plasma membrane (15), it appears that the uptake of trypan blue is a more sensitive index of damage.

Macrophage morphology. As expected (4), exposure to amiodarone had profound effects on macrophages by increasing four times the area covered by inclusion bodies and by >27 times the area covered by vacuoles (Figs. 5–7).

Considering the formation of inclusion bodies, MDEA and dronedarone increased significantly the surface area covered by inclusion bodies (Figs. 5–7). DDEA and B2-O-EtOH did not induce significant changes with respect to control cells, but B2-O-EtOH caused the formation of less inclusion bodies than DDEA (Figs. 5–7). Thus, amiodarone, MDEA, DDEA, and B2-O-EtOH have a progressively smaller ability to induce the formation of inclusion bodies.

Considering the formation of vacuoles, dronedarone had the strongest effect among the substances tested, increasing the surface area by >37 times. Besides dronedarone, amiodarone metabolites also had a significant effect on the formation of vacuoles but showed dramatic differences in potency. In fact, the ability to induce the formation of vesicles appears to rank in the following order: amiodarone > MDEA >> DDEA > B2-O-EtOH (all significantly different from each other by ANOVA) (Fig. 7). Interestingly, some analogs that had no effect on trypan blue exclusion and release of LDH, like B2-O-Et and KB-130015, caused a small but significant increase of the area covered by vesicles (Fig. 7).

Degradation of SP-A by alveolar macrophages. Amiodarone inhibited the degradation of SP-A by alveolar macrophages, the effect starting to be significant at a concentration of 10 μM (P < 0.05, ANOVA) (Fig. 8). MDEA, DDEA, MeAMI, and dronedarone inhibited the degradation of SP-A to the same extent of amiodarone (Fig. 8). B2-O-EtOH, B2-O-AcOH, and KB-130015 also inhibited the degradation of SP-A but did so to a lesser extent and without producing the clear sigmoid

![Fig. 4. Effect of analogs of amiodarone on release of lactate dehydrogenase (LDH) by alveolar macrophages. Analogs are in order of decreasing hydrophobicity from top to bottom. Data (means ± SE) represent % of cell LDH released per hour, after subtraction of LDH released by control cells. N = 6. *Different from amiodarone at the same concentration by ANOVA (P < 0.05).](http://ajplung.physiology.org/ by 10.220.33.6 on June 20, 2017)
Fig. 5. Effect of amiodarone metabolites and analogs on the morphology of alveolar macrophages by light microscopy. Bars = 10 μm.
Fig. 6. Effect of amiodarone metabolites and analogs on the morphology of alveolar macrophages by transmission electron microscopy. Arrowheads: inclusion body; arrows: vacuole. Bars = 1 μm.
Uptake and degradation of SP-A administered through the airways. In control animals, 3 h after the tracheal instillation of $^{125}$I-SP-A, the total radioactivity was evenly distributed between airways and lung tissue, and degradation products were present, especially in the airways (Figs. 9 and 10). Amiodarone inhibited the degradation of SP-A after the uptake, with the consequence that less degradation products were formed and nondegraded SP-A accumulated into lung tissue (Figs. 9 and 10). Dronedarone had an effect comparable with that of amiodarone, causing the accumulation of large amounts of nondegraded SP-A into lung tissue (Figs. 9 and 10). KB-130015 had a much smaller effect, causing a small accumulation of nondegraded SP-A in lung tissue (Figs. 9 and 10).

**DISCUSSION**

MDEA, DDEA, and B2-O-EtOH are metabolites of amiodarone of decreasing hydrophobicity produced by sequential modifications of the diethylaminoethoxy group (Fig. 2). Although the toxicity of MDEA is known in part (5), the effects of DDEA and B2-O-EtOH have never been studied.

We found that the toxicity of MDEA toward alveolar macrophages is greater than that of amiodarone according to trypan blue exclusion and the release of LDH. On the other hand, the toxicity of MDEA is similar to that of amiodarone, considering the effects on the degradation of SP-A and the formation of inclusion bodies, and is smaller than that of amiodarone, if one
looks at the formation of vacuoles. Deethylation of MDEA to DDEA dramatically decreases the ability to cause the formation of vacuoles in the cytoplasm of alveolar macrophages, whereas the rest of the toxicity profile remains unchanged. Deamination of DDEA reduces the toxicity further, since the resulting metabolite (B2-O-EtOH) has no effect on trypan blue exclusion and the release of LDH, has modest effects on morphology, and retains some ability to inhibit the degradation of SP-A. In addition to an improvement of the toxicity profile, the deamination of DDEA could have a further positive effect by influencing pharmacokinetics, since the plasma of patients receiving amiodarone contains measurable amounts of MDEA and DDEA, whereas B2-O-EtOH remains under the limit of detection, possibly because it is quickly eliminated by conjugation (unpublished observations). Thus MDEA, DDEA, and B2-O-EtOH rank in order of decreasing toxicity toward alveolar macrophages, indicating that dealkylation and deamination of the diethylaminoethoxy group are important steps in the biotransformation of amiodarone. The crucial role of this lateral group in the toxicity toward macrophages also emerges from the study of analogs. In fact, the deletion of the diethylaminoethoxy group of amiodarone generates B2, an analog devoid of all toxic effects considered in this study. Because B2 contains the diiodobenzoyl moiety and the benzofuran moiety with its associated butyl group (Figs. 1 and 2), it appears that these parts of the amiodarone molecule play no direct role in the toxicity toward alveolar macrophages. This finding contrasts sharply with the results of studies exploring the mechanism of amiodarone toxicity toward the liver (19). Those experiments showed unambiguously that the benzofuran moiety is responsible for liver toxicity, suggesting that different

Fig. 8. Effect of analogs of amiodarone on the degradation of surfactant protein A (SP-A) by rabbit alveolar macrophages. A: amiodarone metabolites and analogs containing an amine function. B: other metabolites and analogs. Data are % of SP-A degraded by control cells (means ± SE); n = 3–7. *Greater, #smaller than amiodarone at same concentration by ANOVA.
parts of the amiodarone molecule could be toxic toward specific cell types.

Amiodarone interferes with various cell functions and structures, such as late steps of endocytosis (4), the catabolism of proteins and lipids (4, 14), mitochondrial integrity and energy metabolism (13), calcium homeostasis (8, 12), ion pumps (11), and the distribution of receptors among different cell compartments (6, 20). From the evidence presented here, it appears that amiodarone metabolites may also have a plethora of effects since they impair the integrity of the plasma membrane, interfere with the degradation of SP-A, which normally is taken up by macrophages and degraded into lysosomes (4), and impact on the turnover of cell organelles, as indicated by the accumulation of vesicles and multilamellar inclusion bodies.

The nitrogen of the diethy laminoethoxy group appears to play an important role in toxicity toward alveolar macrophages because analogs and metabolites containing it rank at the top of the toxicity scale (amiodarone, MDEA, DDEA, MeAMI, dronedarone) and because its removal during amiodarone metabolism dramatically decreases adverse effects toward macrophages. The presence of a tertiary nitrogen is typical of lysosomotropic amines that, in their neutral form, enter the lysosomes by diffusion but then, in the acidic lysosomal milieu, become insoluble and accumulate to high levels, causing the osmotic swelling of lysosomes (9). Thus it is tempting to speculate that some of the effects of amiodarone might be due to interference with the lysosomes by this mechanism. It is worth noting, however, that lysosomotropic amines with vacuolegenic ability, like methylamine and chloroquine, work at millimolar concentrations (1), whereas amiodarone and its metabolites work at micromolar concentrations. Furthermore, we find that during amiodarone degradation, the greatest decrease in the vacuoligenic ability is due to deethylation of MDEA rather than to deamination of DDEA (Figs. 5–7). Finally, we show that analogs lacking the amine function retain some ability of generating vacuoles (like B2-O-EtOH, B2-O-Et, and KB-130015) and can still interfere with the degradation of SP-A (B2-O-EtOH, B2-O-AcOH, and KB-130015). Thus the role of the tertiary nitrogen of amiodarone in macrophage toxicity remains to be fully defined.

One aim of this investigation was to analyze the effects on alveolar macrophages of two amiodarone derivatives with proven antiarrhythmic activity, dronedarone and KB-130015, and to compare them with those of amiodarone. Our results indicate that, at equimolar concentrations, dronedarone has a toxicity greater than or equal to that of KB-130015. The overall picture that emerges from this study can be summarized as follows: 1) amiodarone metabolites MDEA, DDEA, and B2-O-EtOH rank in order of decreasing toxicity toward alveolar macrophages, indicating that dealkylation and deamination of the diethy laminoethoxy group are important mechanisms of detoxification; 2) dronedarone and KB-130015 have respectively greater and smaller toxicity toward alveolar macrophages than amiodarone; and 3) the benzofuran moiety, which is toxic to liver cells, is not directly toxic toward alveolar macrophages.

It is important to note that these conclusions are based on a limited number of effects observed on isolated cells after incubation with drugs present at concentrations that may or may not be reached in vivo. Furthermore, many of the most important effects of amiodarone, such those on mitochondrial function, on the traffic of lysosom al enzymes, on calcium homeostasis, and on the regulation of apoptosis (2, 10) were not examined in the present investigation. Finally, it should be considered that effects interpreted here as toxic toward alveolar macrophages, such as the interference with the traffic of vesicles, could represent a desirable end point in other respects, like the recently discovered antifungal activity of amiodarone (12).

ACKNOWLEDGMENTS

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GRANTS

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REFERENCES


