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Cationic Amphiphilic Drug-Induced Phospholipidosis

WILLIAM H. HALLIWELL

Schering-Plough Research Institute, P.O. Box 32, Route 94 South, Lafayette, New Jersey 07848

ABSTRACT

Phospholipidosis, a phospholipid storage disorder, defines an excessive accumulation of intracellular phospholipids. Phospholipids are structural components of mammalian cytoskeleton and cell membranes. The metabolism of this essential cell component is regulated by the individual cell and may be altered by drugs that interact with phospholipids or the enzymes that affect their metabolism. Xenobiotics or their metabolites that induce phospholipidosis include a wide variety of pharmacologic agents, including antibacterials, antipsychotics, antidepressants, antiarrhythmics, antianginals, antimalarials, anorexic agents, cholesterol-lowering agents, and others. Each of these drugs shares several common physiochemical properties: hydrophobic ring structure on the molecule and a hydrophilic side chain with a charged cationic amine group, hence the class term cationic amphiphilic drugs (CADs). This paper reviews the phospholipid metabolism, physiochemical characteristics of CADs, specificity of phospholipidosis in animals and humans, functional effects of phospholipidosis, interaction of CADs with biologic membranes and lysosome metabolism, influence of CADs on phospholipases and phospholipid synthesis, and a proposed mechanism for induction of phospholipidosis in the lung. In human risk assessment, investigators should consider the many factors in evaluating a drug that induces phospholipidosis in animals. These include: the therapeutic class of drug, presence of active metabolites, tissue or organ selectivity in animals and humans, influence of concurrently administered drugs, reversibility of effect, and other factors that increase or decrease the induction of phospholipidosis. Generalities regarding the etiology, incidence, and effect of the drug on a specific host may not be made. Each drug must be evaluated separately to identify the risk when administered for therapeutic effect in humans.

Keywords. Phospholipidosis; cationic amphiphilic drugs; phospholipid storage disorder; foamy alveolar macrophages; lysosomal lamellar bodies

INTRODUCTION

In 1996, Greselin reported that a cholesterol synthesis-inhibiting drug trans-1,4-bis(2-chlorobenzylaminomethyl) cyclohexane dichloride (AY-9944), induced increased numbers of foam cells in the pulmonary alveoli of rats (19). In 1971 there was the description of diethylaminoethoxyhexestrol (DH)-induced foam cell lipidosis in humans (85). Since that time, there have been many reports of xenobiotic-induced phospholipid storage disorders. More than 50 cationic amphophilic drugs (CADs) administered to laboratory animals, humans, and cultured cells result in the induction of a generalized lipid storage disorder in many tissues of the body (39,47,48,50). Phospholipidosis describes the excessive accumulation of phospholipids in affected cell lysosomes that acquire a multilamellar morphologic appearance. Phospholipids are essential components of cell membranes. They contain a greater proportion of polar groups and are, therefore, partly soluble in water and partly soluble in nonpolar solvents. The bilayer of such polar lipids has been regarded as a basic structure in biologic membranes. Their synthesis and metabolism are regulated by individual cells and tissues. Metabolic dysfunction associated with, or induced by, genetic disorders may produce lysosomal storage of phospholipids, such as Niemann–Pick and Tay–Sachs diseases (12). However, xenobiotic drugs and chemicals, as well as hormones, cofactors, and other agents, may alter the metabolism of the cell and result in phospholipidosis (39).

*Address correspondence to: Dr. William H. Halliwell, Schering-Plough Research Institute, P.O. Box 32, Route 94 South, Lafayette, New Jersey 07848.

Phospholipidosis may be induced by the direct interaction of xenobiotics with intracellular phospholipids or by the action of xenobiotics on the synthesis and metabolism of phospholipids (36,39). The intracellular phospholipid content may increase to many times the normal content of the cell (23). Many factors contribute to the development of phospholipidosis including structural formula of the CAD, intra- and interspecies susceptibility, dose, duration of dosing (exposure) and mechanism of action of the CAD on the metabolism of specific phospholipids (39).

The induction of phospholipidosis by the exposure to CADs results in the possible accumulation or retention of phospholipids in virtually every tissue or organ in the body. Commonly, excessive accumulation is seen in the lung, liver, brain, kidney, ocular tissues, heart, adrenal glands, hematopoietic tissue, and circulating lymphocytes, but virtually all tissues of the body are capable of excessive phospholipid accumulation (3,32,47,50,55). Several excellent reviews of phospholipidosis induction by CADs have been published. (27,29,37,39,51,52,64). These publications span 20 yr of investigations into the etiology, mechanisms, effects, and reversibility of phospholipidosis in animals and its relevance in assessing human risk assessment.

There is a wide variation in the species susceptibility for animals and humans to phospholipidosis. It is not uncommon to recognize different organ or tissue susceptibility and severity when comparisons are made between animals and humans. These features are particularly relevant in human risk assessment. The use of cell culture has become a useful tool in the evaluation of the potential

for a xenobiotic to induce phospholipidosis and to investigate phospholipid metabolism (2,69).

I. What Is Phospholipidosis?

Phospholipidosis is the excessive intracellular accumulation of phospholipids induced by the short-term or chronic administration of CADs. The induction time may be a few days to several months, depending on the affinity of the CAD for susceptible cells (25,34). In cell cultures, phospholipids can accumulate intracellularly and induce lysosomal lamellar body formation, within only a few hours of exposure (69).

In the normal lung, production of surfactant is a dynamic process. Type II pneumocytes synthesize surfactant in lamellar bodies and secrete it into the alveolar space by exocytosis. Surfactant is taken up by pinocytic action of alveolar macrophages, processed, and then extruded into the alveolar space. Some is then taken up by the type II pneumocytes and recycled (61,64). Although phospholipidosis may occur in almost any tissue in the body, the lung and the alveolar macrophages are usually prominent in their response to administration of most CADs.

The experimental lung lesion is characterized by the excessive accumulation of foamy alveolar macrophages, mononuclear cells, and amorphous material in the alveolar spaces of the lung (25,26,52). In pulmonary phospholipidosis, there is an increased amount of phospholipid in the lung tissue and/or alveolar macrophages. Lungs from rats treated intraperitoneally with chlorphentermine (30 mg/kg) for 4 wk had: 1) marked accumulation of alveolar macrophages in the alveoli; 2) the alveolar macrophages were heterogeneous in size, with many up to 10 times normal volume; and 3) the alveolar macrophages became engorged with lysosomal lamellar bodies and granular material. Initially the lysosomes swell, some fragment, and others develop a lamellar pattern. The cell then becomes filled with lysosomal lamellar bodies and amorphous granular material derived from deterioration of the lysosomal lamellar bodies. Alveolar macrophages disintegrate and distribute the granular material to the extracellular space (65). The increased total phospholipids in the alveolar macrophages of rats treated with chlorphentermine are composed of phosphatidylcholine, sphingomyelin, phosphatidylserine, and phosphatidylethanolamine (65). The cellular changes induced by most CADs are generally reversible, but the effects on tissues do not return to control levels at the same time (65).

II. Drugs That Induce Phospholipidosis

There are over 50 known CADs that induce phospholipidosis in one or more tissues in the body and they include many different therapeutic classes of drugs. Some of the classes of drugs are antidepressants, antiarrythmics, antianginals, antibacterials, antimalarials, anorexic agents, antipsychotics, cholesterol-reducing agents, and others (4,25,32,47,48,64) (Fig. 1).

It is important to recognize that each of these xenobiotics possibly has a different species and tissue selectivity, affinity for phospholipids, metabolism or metabolites, and other biochemical or structural differences so that each will induce a slightly different manifestation of phospholipidosis.

Despite the diverse pharmacologic activity, therapeutic indications, diversity of tissue selectivity, and distinct manifestations of phospholipidosis that each of these CADs can induce in different species of animals, they do share several common physiochemical similarities. The physiochemical properties most commonly shared by CADs are a hydrophobic ring structure on the molecule and a hydrophilic side chain with a charged cationic amine group. These two structural entities provide the amphiphilicity that is common to these drugs, and therefore they are identified as cationic amphophilic amines (30,39).

The hydrophobic structure enhances the molecule's ability to pass through plasma membranes when they are not ionized. The ionized form of the molecule tends to remain with the membrane and contribute to membranous changes (30,39,76). Thus it can be visualized that cell membrane phospholipids and their charged ionic groups monitor CAD penetration and bonding in cells (39). The addition of a halogen group to the hydrophobic ring seems to enhance membrane penetrability (18,75).

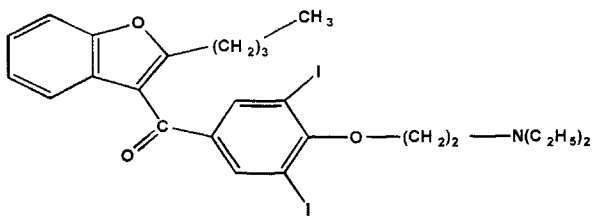
The diversity of the therapeutic activity of CADs is dependent on their effect on membrane composition, transition temperature, membrane fluidization, receptor site mediation, and other functions not yet well understood (9,44,46).

Many of the antiarrhythmics, B blockers, and antipsychotics affect ion channels and receptors (46,87). Amiodarone, an antiarrhythmic drug, inhibits Na^+ penetration of cell membranes and also affects Ca^{2+} movement (77). Propanolol is a beta-adrenergic blocker that is primarily receptor site mediated (60). Neuroleptics and antipsychotics, for example promazine and chlorpromazine, are lipid soluble and influence membrane permeability (74,76).

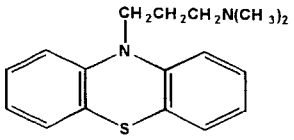
Disobutamide induces clear cytoplasmic vacuoles that are indicative of intracellular drug storage and concentric lamellar bodies in multiple tissues and organs (68,70). It has been proposed that the unique structure of this CAD, with two basic amines on the hydrophobic chain, induces both clear cytoplasmic membrane-bound vacuoles that are storage sites of drug (disobutamide) and also induces lysosomal lamellar bodies typical of phospholipidosis (68,70). Chloroquine (Fig. 1) also contains two basic amines on the hydrophilic side chain and induces clear cytoplasmic vacuoles and lysosomal lamellar bodies (39).

Two major concepts have been proposed for the mechanism of CAD-induced phospholipidosis. The first proposes that CADs bind to phospholipids and the complex becomes more resistant to degradation by phospholipases (30,48). Secondly, is the hypothesis that CADs directly inhibit the enzymes responsible for phospholipid catabolism (41). It is plausible that both mechanisms, or various combinations of them, are responsible for the varied response seen in phospholipidosis in animals and humans (30).

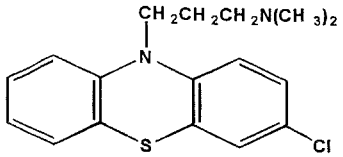
Amiodarone **Antiarrhythmic**



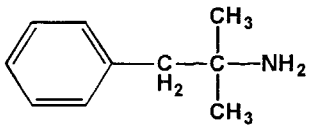
Promazine **Antipsychotic**



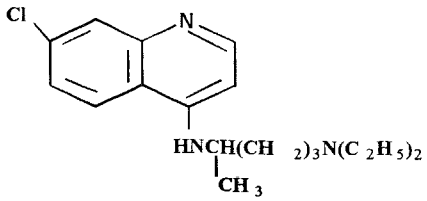
Clorpromazine **Antipsychotic**



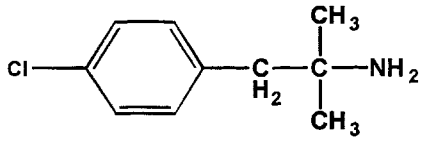
Phentermine **Anorexic**



Chloroquine **Antimalarial**



Chlorphentermine **Anorexic**



Disobutamide **Antiarrhythmic**

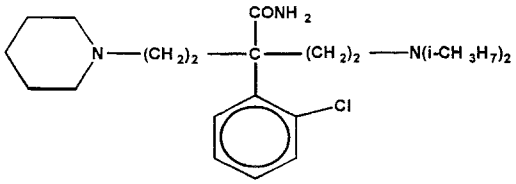


FIG. 1.—Structural formulas and therapeutic use of various amphophilic drugs.

III. SPECIFICITY OF PHOSPHOLIPIDOSIS: ANIMAL SPECIES, TISSUE, AND AGE SUSCEPTIBILITY

The distribution of specific phospholipids in various tissues is dependent on the structure and function of each tissue and also the species and age of the animal. These factors and others determine the incidence and severity of phospholipidosis induced by CADs. Recognition of the ionic and hydrophobic interactions of CADs with phospholipids or phospholipases is important in recognizing the diversity of response of these molecules (30,49). Chlorphentermine reacts more vigorously with phospholipids that have polar ionic moieties; hydrophobic interactions are minor (30). Chlorphentermine binds mainly to phosphatidylcholine and charged polar lipids. Surfactant in the lung contains high levels of disaturated phosphatidylcholine, therefore, it is not surprising that cholorphentermine produces dramatic pulmonary phospholipidosis in rats (30,66,79).

In contrast, amiodarone binds most vigorously to the hydrophobic moiety of phospholipids, and ionic interactions of the polar moiety are minimal (29). Treatment with amiodarone produces a significant elevation of phosphatidylcholine particularly in alveolar macrophages and type II pneumocytes (17). Amiodarone also induces phospholipidosis in the liver with large increases in phosphatidylserine and phosphatidylethanolamine (62,86). It is noteworthy that amiodarone does not induce significant numbers of lysosomal lamellar bodies in the hepatocyte cytoplasm but does induce cytoplasmic vacuoles (43,62).

Gentamicin is a CAD with a different affinity; it pro-

duces phospholipidosis predominantly in the kidney. Gentamicin, an aminoglycoside antibiotic, is predominantly excreted by glomerular filtration and then binds to the brush-border membranes of the proximal tubule epithelial cells where it is adsorbed by endocytosis. Subsequently, it accumulates in lysosomes of the proximal tubule epithelium (1,16,84). Analysis of homogenate and lysosomal fractions of kidney cortex reveals increases in concentration of total renal phospholipids including: phosphatidylserine, phosphatidylcholine, and phosphatidylinositol. These changes are accompanied by a significant reduction in phospholipase C, an enzyme with a high affinity for phosphatidylcholine and other phospholipids (35,84).

The role of CADs in the alteration of cell-to-cell signaling has not been well defined; however, the alterations in levels of phosphatidylinositol, phosphatidylcholine, and others point to alterations in signal transduction (39,73).

Even within species, there can be vast differences in the manifestation of phospholipidosis. McCloud et al have investigated the accumulation of amiodarone and its metabolites and its propensity to induce phospholipidosis in Fisher 344 rats and Sprague Dawley rats (56). In these studies, amiodarone was administered at 100 mg/kg/day for 1 wk or 4 wk. The results from these two studies were similar—phospholipidosis was induced in the lung tissues of the Fisher 344 rats but not significantly in the Sprague Dawley rats. It was concluded the strain differences were related to the dispositional location of the drug (56).

The role that age contributes to CAD-induced morphologic and metabolic response has also been investigated. Newborn rats treated with chlorphentermine or chlorcyclizine for 1 wk did not develop hypertrophic vacuolated alveolar macrophages; however, adult rats treated with the same CADs at the same dose and for the same duration did develop hypertrophic vacuolated alveolar macrophages containing lysosomal lamellar bodies (33,34).

There is significant evidence of pharmacologic manipulation of drug-induced phospholipidosis. Chlorphentermine-induced phospholipidosis of alveolar macrophages was reduced in incidence and severity when phenobarbital was concurrently administered (33). Several related studies demonstrated that the concurrent administration of phenobarbital with chlorphentermine reduced the accumulation of phospholipids in affected organs. These findings were attributed to the induction of specific drug-metabolizing enzymes by phenobarbital (33,36,80).

It is apparent that many factors affect the ability of CADs to induce phospholipidosis. Included are species and strain differences, specific tissue affinity, structural and biochemical relationship, concurrent drug administration, age of the patient, metabolic rate of the drug and its metabolites, and pharmacokinetics of each of these components. In risk assessment to humans, these variables must be assessed for each specific phospholipidosis-inducing drug.

IV. Functional Effects of Phospholipidosis

The dramatic morphologic changes induced in the lungs of some animals by CAD administration, and the known affinity of pulmonary tissue for CADs, has prompted investigations into the functionality of these organs (14). Camus et al investigated the changes in pulmonary respiratory function in rats treated with chlorphentermine. They reported that despite massive induction of pulmonary phospholipidosis, there were only minor effects on lung function (5,6).

Amiodarone is an iodinated antiarrhythmic drug that is reported to induce generalized phospholipidosis in several animal species as well as humans (21). It has been suggested that amiodarone interferes with phospholipase A1 and A2 activity in the degradation of phospholipids (22,24,47). In humans and hamsters and other animals, administration of amiodarone is associated with generalized phospholipidosis, pulmonary fibrosis, and increased hepatic density (7,8). However, investigators are unsure if the phospholipidosis induces these changes in vital organs, or if the iodinated molecule depositing in these sites is responsible for some of the changes, or perhaps there is some other combination of factors inciting these changes (67).

There are some situations in which phospholipidosis has been shown to have a definite functional effect. A common feature of CAD administration is the presence of lysosomal lamellar bodies in lymphocytes of some species. Mice treated with chlorphentermine *in vivo* had a significantly depressed ability to generate a delayed hypersensitivity response or to produce antibody-secreting cells against *de novo* antigen (71,72). Mouse splenic lymphocytes exposed to 10^{-7} M chlorphentermine for 3 days *in vitro* had a significantly depressed blastogenic response to the mitogens phytohemagglutinin, concanavalin A, and lipopolysaccharide (71,72).

V. Interactions of CADs with Biologic Membranes

The biologic or pharmacologic activity of xenobiotics may occur at many sites, however, one of the more important is the interaction with biologic membranes. In this context, drugs must penetrate the lipid bilayer and thus they affect the physiochemical properties of the lipid bilayer (44).

The presence of a halogen group on the hydrophobic portion of a molecule in some cases enhances the biologic and pharmacologic effects of the molecule, as in the comparison of phentermine and chlorphentermine or promazine and chlorpromazine (Fig. 1). The halogen group on the hydrophobic moiety increases the lipophilicity and the phospholipidosis-inducing capacity of these CADs (75,76).

The binding of CAD molecules to the hydrophobic and hydrophilic moieties of the phospholipids may affect the rate of metabolism of these molecules (39). The role that the structure of the CAD and its interaction with biologic membranes and other active sites on cells is complex and merits further investigation (11,39).

VI. The Effect of CADs on Lysosome Metabolism

Alterations in lysosome metabolism by CADs and the development of lysosomal lamellar bodies are intimately related to the development of phospholipidosis. Several investigators have shown that xenobiotics are weak bases have an affinity for lysosomes (13,83). CADs that are basic and have a pKa higher than 7–8 preferentially concentrate in the lysosomes (48). It follows then that CADs move toward and localize in lysosomes that contain anionic lipids (39).

Some prominent CADs, such as amiodarone, have a lesser affinity for lysosomes (20). Amiodarone contains only one basic amine in the hydrophilic portion of the molecule and has limited reaction with polar phospholipids and storage in lysosomes yet does induce lysosomal lamellar bodies (39).

The effect phospholipidosis has on lysosomal function has been addressed by several investigators. Lullmann-Rauch and Watermann investigated lysosomes that had been converted to lysosomal lamellar bodies in renal epithelial cells and in hepatocytes of rats. These lysosomes retained their ability to fuse with autolysosomes and/or autophagosomes (53).

VII. Inhibition of Phospholipases

The mechanisms that produce phospholipidosis are difficult to define because there are so many xenobiotics that when administered at the appropriate dose, duration, and multiple other factors already discussed can result in the accumulation of phospholipids and development of lysosomal lamellar bodies. It is simple to speculate on the factors that result in excessive phospholipids in the lysosomes:

1. CADs bind to phospholipids and form complexes in lysosomes that become lysosomal lamellar bodies. These lysosomal lamellar bodies are variably resistant to phospholipase enzyme activity. Concerning recovery, the complexes are unstable and become more susceptible to phospholipase activity after cessation of CAD administration (29,31,48,50). Recovery from phospholipidosis after drug discontinuance may be a couple of weeks to months depending on the CAD administered and multiple host factors.
2. The second theory implies that CADs inhibit phospholipase activity. This theory has been investigated by many researchers (22,24,40,42,78). Critics of this theory state that most tests are conducted *in vitro* and it is difficult to detect if the CAD binds to the phospholipid or if it inhibits the phospholipase in the incubation medium. A good discussion of these vagaries of activity is presented in a paper by Kodavanti and Mehendale (39).

Probably the best support for the theory that CADs inhibit phospholipase activity is a series of *in vitro* studies with amiodarone (24,54).

The mechanism of phospholipidosis remains unsettled. The wide spectrum of xenobiotics that induce phospholipidosis, the difficulty in isolating specific substrates and enzymes, and the variable recovery times after withdrawal remain as obstacles to a comprehensive explanation. In the evaluation of risk assessment, individual drugs should be investigated and assessed with more certainty than the entire class of CADs that induce phospholipidosis.

VIII. Phospholipid Synthesis—The Effects of CADs

Some researchers have also investigated the plausibility that administration of CADs can increase the synthesis of phospholipids resulting in phospholipidosis. Some *in vitro* and *in vivo* studies do support this theory. In cultures of skin fibroblasts, chloroquine stimulated phospholipid and cholesterol synthesis (10). Chlorpromazine has also been reported to increase cellular synthesis of phospholipids *in vitro* (45).

It is apparent from these observations and those in the previous section that CADs do have an influence on phospholipid metabolism. The influence may depend on binding to phospholipids and inhibiting breakdown, influencing enzymes, resulting in reduced catabolism or influencing the synthesis of phospholipids. In each case, researchers have demonstrated by *in vivo* and/or *in vitro* techniques support for their theories. However, the vast array of CADs, the species, strain, and age variation, and a host of other factors suggest that each CAD and host have unique interactions and that in risk assessment for humans one should focus on the molecule under investigation and conduct the studies necessary to provide answers relevant to human risk.

IX. The Mechanism of CAD-Induced Phospholipidosis

Induction of phospholipidosis has been attributed to a multitude of factors. There are features that are common to most of these pathways at the organ, tissue, cell, enzyme system, and molecular level. From these features and others, Joshi and Mehendale (30) have proposed the following generalized mechanism of phospholipidosis in the lung (Fig. 2).

X. Phospholipid Metabolism and Effects on Cell Function

The various cellular lipids and their influence on cell metabolism have been investigated for many years. These products influence regulation of cell function, cell-to-cell signaling, cell growth, receptor sites, and other membrane-associated events. Most of these activities appear to be active at the molecular level and vary with the CAD under investigation.

The effects of CADs on receptor-mediated events have been reported to be attributed to influences of CAD–membrane interactions (41). The role of phosphatidylinositol, arachadonic acid, prostaglandins, interleukins, platelet-activating factors, and others in cell-to-cell signaling is becoming a feature that can be investigated and identified and utilized in drug development (15,38,81).

The role of CADs and their effects on cell metabolism through alterations in phospholipids remain to be identified and understood, but it is certain that they are able to influence a wide variety of cell-to-cell interactions (31). Protein kinases catalyze phosphorylation reactions but are influenced by endogenous regulatory products re-

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