MINIREVIEW

Drug-Induced Phospholipidosis: Are There Functional Consequences?

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Phospholipidosis induced by drugs with a cationic amphiphilic structure is a generalized condition in humans and animals that is characterized by an intracellular accumulation of phospholipids and the concurrent development of concentric lamellar bodies. The primary mechanism responsible for the development of phospholipidosis is an inhibition of lysosomal phospholipase activity by the drugs. While the biochemical and ultrastructural features of the condition have been well characterized, much less effort has been directed toward understanding whether the condition has adverse effects on the organism. While there are a few cationic amphiphilic drugs that have been reported to cause phospholipidosis in humans, the principal concern with this condition is in the pharmaceutical industry during preclinical testing. While this class of drugs should technically be referred to as cationic lipophilic, the term cationic amphiphilic is widely used and recognized in this field, and for this reason, the terminology cationic amphiphilic drugs (CADs) will be employed in this Minireview. The aim of this Minireview is to provide an evaluation of the state of knowledge on the functional consequences of CAD-induced phospholipidosis.

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Overview of Cationic Amphiphilic Drug- (CAD) Induced Phospholipidosis

Numerous drugs containing a cationic lipophilic structure are capable of inducing a phospholipidosis in cells under conditions of *in vivo* administration (1–3) or *ex vivo* incubation (4–7). Drugs with this type of structure typically contain a hydrophilic domain consisting of one or more primary or substituted nitrogen groups positively charged at physiological pH, and a hydrophobic domain consisting of an aromatic and/or aliphatic ring structure, which may be substituted with one or more halogen moieties (Fig. 1). While not technically correct, this class of drugs has been referred to historically as cationic amphiliphic drugs (CADs), and for the sake of name recognition, this designation will be used throughout this Minireview. If the accumulation of phospholipids is extensive, it can be characterized biochemically (8–13); however, morphological/ultrastructural changes are the hallmark feature of this disorder (1). When examined light microscopically, cells may appear vacuolated, although this is not always evident. The characteristic feature of CAD-induced phospholipidosis, detected electron microscopically, is the appearance within the cell of unicentric or multicentric lamellated membranous inclusions, hereafter termed lamellar bodies. The membranous structures may be tightly packed or may have clear or granulated matrix associated with the lamellae. While cystalline-like and reticular membranous patterns have been described in response to CADs, these patterns are extremely rare.

CAD-induced phospholipidosis is characterized by four principal features (1): excessive accumulation of phospholipids in cells; ultrastructural appearance of membranous lamellar inclusions, predominantly lysosomal in origin; accumulation of the inducing drug in association with the increased phospholipids; and reversibility of alterations af-

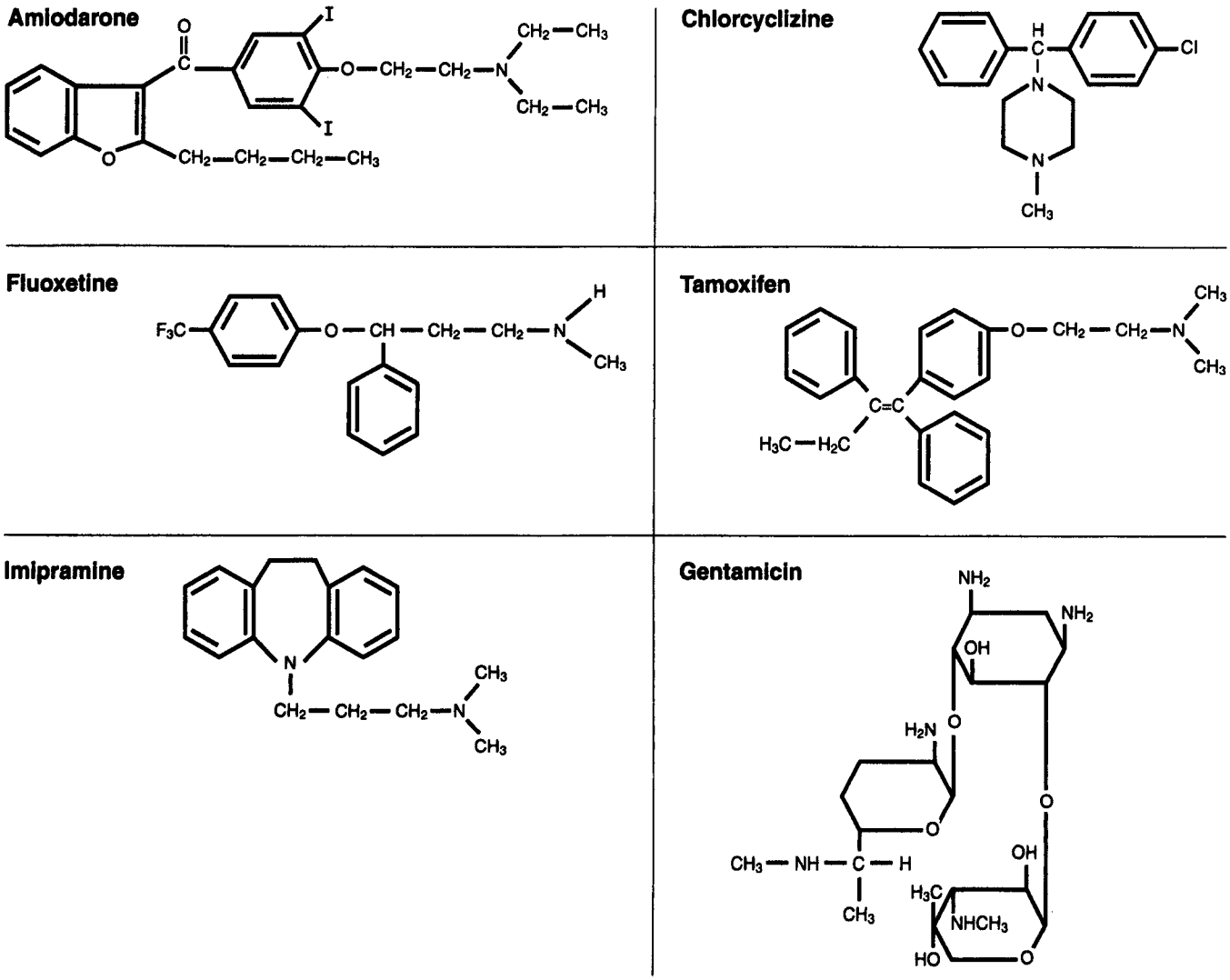

Figure 1. Structures of representative drugs that induce lamellar bodies in cells *in vivo* or *ex vivo*. Amiodarone, fluoxetine, imipramine, chlorcyclizine, tamoxifen, and gentamicin are considered cationic amphiphilic (lipophilic) drugs because each has a hydrophobic region containing a substituted or unsubstituted aromatic or aliphatic ring structure and a hydrophilic region consisting of one or more primary or substituted nitrogen groups that are positively charged at physiological pH.

ter discontinuance of drug treatment. In this report, a brief overview of CAD-induced phospholipidosis will be presented. Numerous review articles are available that provide a list of CADs that cause phospholipidosis and describe features of this condition, including the ultrastructural characteristics of the lysosomal lamellar bodies (1–3, 14–26).

In what is believed to be the first account of CAD-induced phospholipidosis, Nelson and Fitzhugh (27) reported the induction of foamy macrophages in rats by long-term treatment with chloroquine. At the time of the publication, it was not recognized that the response was a phospholipidosis, as this phenomenon was unknown, but subsequent research has confirmed that foamy macrophages develop as a result of the accumulation of phospholipids within the cell and are a common response to CADs (14, 28). Since that time, hundreds of reports have appeared on the topic of CAD-induced phospholipidosis. Over 50 clinically relevant and experimental CADs have been reported to induce phospholipidosis following *in vivo* administration and/or under *ex vivo* conditions. This information is available in the peer-reviewed literature, and it can also be obtained through freedom of information in the Summary Basis for Approval documents prepared by the Food and Drug Administration. The development of phospholipidosis during preclinical testing in animals is a recognized problem in the pharmaceutical industry and can delay or abort the development process. For this reason, and because it has been several years since the topic of the toxicological significance of CAD-induced phospholipidosis has been reviewed (7), the present Minireview was prepared.

In many animal studies, phospholipidosis has been observed following administration of the CAD generally at levels far in excess of that used clinically. As far as the authors are aware, only amiodarone (11, 29), perhexiline (30), fluoxetine (31), gentamicin (32), and 4,4′-diethylaminoethoxyhexestrol (33) have been reported to induce the condition in humans.

The induction of phospholipidosis by CADs is a dose-dependent process, with its development being directly proportional to the accumulation of CAD in the cell or tissue. The phospholipids in cells may be of intracellular or extracellular origin. The phospholipidosis is a generalized phenomenon in that the lamellar bodies may occur in virtually any tissue, with the lungs and liver being common targets. No species or age group appears to be excluded from their induction. Different species, strains within species, and age groups may respond dissimilarly, however, to the same CAD (20, 34–36). Within the same species, different CADs may exhibit different tissue specificity, qualitatively and quantitatively, for the induction of the condition. Consequently, the response of a given species to a particular CAD is both qualitatively and quantitatively unpredictable (20).

It is reasonable to believe that the pharmacokinetic properties of a CAD are responsible for these characteristics. Because of the variability among species, or a particular strain within a species, in the induction of phospholipidosis, caution must be exercised in predicting the susceptibility of humans based on results in animals. The role of rat strain and other confounding factors in the interpretation of toxicity data have been reviewed (37, 38).

Within cells, CADs accumulate principally in lysosomes (18, 39). There is consistent evidence that CADs inhibit lysosomal phospholipase activity (18, 40), resulting in the accumulation of one, but more commonly, several classes of phospholipids within the organelle. This inhibition appears to be the primary method by which CADs induce phospholipidosis, and there are two theories as to how this occurs. The first involves CADs binding to hydrophobic and hydrophilic moieties of phospholipids, resulting in complexes that are undigestible by lysosomal phospholipases (4, 39). The second theory involves direct inhibition of lysosomal phospholipases by the CADs (41). While both theories may be applicable depending on the CAD and cellular circumstances, evidence is strong that CADs bind to phospholipids (3, 42), and it seems clear that this binding plays an important role in the development of the disorder with most CADs. Additionally, an increase in or redirection of synthesis of phospholipids may play a role in the induction of lysosomal phospholipidosis for some drugs (43), although this mechanism has not been studied extensively. It is likely that the mechanism(s) underlying CAD-induced phospholipidosis is/are not exactly the same for each CAD and is/are more complex than studies have indicated.

Upon termination of drug administration or exposure, the phospholipidosis is reversible with the drug effluxing from the cell, the phospholipid levels returning to normal, and the ultrastructural changes disappearing (7, 39, 44–46). The time course of reversal is dependent on the dissociation rate constant of the CAD from the phospholipid and the elimination rate of the CAD from the tissue. In animal studies, reversal occurs within weeks to a few months depending on these factors. There is little information on the rate of reversal in humans. Amiodarone-induced phospholipidosis is reported to be reversible when the cornea is affected (47); however, in three reports, lamellar inclusions were still present in the liver several months after discontinuation of drug treatment (29, 48, 49).

Functional Consequences of CAD-Induced Phospholipidosis

The prevailing theory is that the phospholipidosis is primarily an adaptive response to CAD exposure rather than a toxic response. In this case, the cell adapts to the drug exposure by sequestering it in the lamellar bodies, thus reducing potential toxicity to intracellular structures. In the process of doing this, however, concentrations of the drug can reach millimolar levels in the lamellar bodies (50).

Studies have failed to definitively show that the presence of CAD-induced phospholipidosis is detrimental to the organism. Isolated cell studies have demonstrated that the presence of the condition may or may not result in changes in pulmonary cellular function, and where this occurs, the consequences to the intact organism are unknown. Additionally, studies involving whole animals have not demonstrated adverse effects attributable to the presence of CAD-phospholipidosis, at least for the lungs. These studies have been summarized previously (2, 17, 51) and will not be reviewed here. The following discussion will expand upon certain studies that highlight what the authors believe are illustrative of our state of knowledge in this field.

Reasor *et al.* (51) administered amiodarone to rats, inducing phospholipidosis in alveolar macrophages. Using this model, a number of pulmonary host defense functions were evaluated. The pulmonary clearance of *Listeria monocytogenes* was not affected by the phospholipidotic condition. Using an *ex vivo* alveolar macrophage culture system, the presence of the phospholipidosis had no effect on the phagocytosis of heat-killed yeast cells, the induction of luminol-dependent chemiluminescence, the spontaneous release of interleukin-6 or tumor necrosis factor-α, or spontaneous and LPS-stimulated release of interleukin-1. In contrast, the LPS-stimulated release of interleukin-6 and tumor necrosis factor-α was enhanced compared with nonphospholipidotic cells. In the context of the functions studied, the induction of pulmonary phospholipidosis appeared not to impair pulmonary host defense processes in rats.

A study by Ferin (52) demonstrated impaired pulmonary clearance of particles in rats treated with the CAD chlorphentermine, but the presence of phospholipidosis in the lungs was not verified.

Sun *et al.* (53) examined the effects of the CAD amiodarone on rat and human hepatocytes, *ex vivo*. They reported that the induction of lamellar bodies preceded a decrease in cell function, which preceded cell death. The results could be interpreted that the induction of lamellar bodies is not directly associated with toxicity, but rather toxicity may result from higher drug concentration in the tissue (tissue overdose effect). However, as the number of lamellar bodies within cells reached high levels, cytotoxic-

ity occurred. Whether this relationship was causal is unknown. Since cytotoxicity occurred at higher drug concentrations in the medium, it is likely that the direct action of the higher drug concentration was responsible for cell injury.

Induction of pulmonary phospholipidosis in rats by amiodarone resulted in an inhibition of phagocytosis of zymosan particles by alveolar macrophages when studied *ex vivo* (54). Oxidative activity of these cells during zymosan stimulation was not altered by the induction of phospholipidosis. These results may be influenced by the fact that the amiodarone-treated animals lost considerable body weight, whereas the controls gained weight.

Long-term treatment of rats with amiodarone resulted in an inhibition of phagocytosis of *Candida albicans* by alveolar macrophages when studied *ex vivo* (55). While the presence of phospholipidosis was not verified in the cells, the treatment protocol was one that had induced the condition in previous studies. In the same study (55), spleen cells exhibited a markedly depressed mitogenic response to phytohemagglutinin stimulation *ex vivo*. There was no evidence presented, however, that phospholipidosis was present in these cells.

Sauers *et al.* (56) reported the induction of phospholipidosis in splenic lymphocytes following the administration of chlorphentermine to mice. Treated mice demonstrated a significantly depressed ability to generate a delayed hypersensitivity response or to produce antibody-secreting cells against *de novo* antigens. The critical process that was sensitive to the drug occurred within the first 10 min of mitogenic stimulation, a time well before lamellar body formation would occur. Others have reported the effects of CADs on various cellular processes *ex vivo*, but have not examined the cells for the presence of lamellar inclusions (57).

Ruben *et al.* (58) reported the absence of pathological change in tissues from dogs and rats in which the administration of disobutamide induced lamellar inclusion bodies and clear vacuoles. This study supports the theory that this condition can exist in tissues without inducing injury as assessed by traditional criteria.

It is recognized that in rats and humans, certain CADs can reversibly induce lamellar inclusions to form in the lens and cornea of the eye (55–61). The physical presence of the structure would be expected to result in impairment in light transmission, although case reports indicate that this rarely occurs (60, 61).

There are studies that suggest CAD-induced phospholipidosis may actually attenuate the pulmonary toxicity of certain materials. Rats with amiodarone-induced pulmonary phospholipidosis showed partial protection against acute and subchronic pulmonary toxicity resulting from the intratracheal instillation of silica (62, 63). Using an *ex vivo* alveolar macrophage system, it was demonstrated that phospholipidotic cells were more resistant to the toxicity of surfactant-coated silica, the form in which silica is present in the alveoli after exposure (62). This result is consistent with the theory that the protective effect of the phospholipidosis to the alveolar macrophages may be due to the ability of amiodarone in the cells to inhibit cellular phospholipases and thus prevent enzymatic digestion of the phospholipid coating the silica. Through this inhibitory action, the toxification of silica would be inhibited and its cytotoxicity and subsequent tissue sequella would be attenuated.

The presence of pulmonary phospholipidosis induced by chlorphentermine offered partial protection against the lethal effects and pulmonary toxicity of inhaled nitrogen dioxide in mice (64). Mice receiving chlorphentermine, and then nitrogen dioxide experienced less terminal bronchiolar hyperplasia and less pulmonary edema, as well as reduced loss of type I cells and reduced increase in type II cells of the lungs than mice receiving only nitrogen dioxide.

The only circumstance where a causal relationship between the presence of phospholipidosis and tissue dysfunction has been suggested is with aminoglycosides and nephrotoxicity. Gentamicin is the best characterized in this regard, yet the results are not definitive. It induces phospholipidosis in renal tissue, and this is associated with renal tubular toxicity, although other cellular changes occur as well (65–68). Inhibition of the development of phospholipidosis inhibits nephrotoxicity (69, 70). Therefore, a cause and effect relationship between gentamicin-induced phospholipidosis and nephrotoxicity has been suggested. Other possible mechanisms involved in gentamicin nephrotoxicity have been reviewed (71). Some of these mechanisms illustrate that the toxic actions of gentamicin and other aminoglycosides on the kidney are probably multifactorial. Because of their polycationic character, gentamicin and other aminoglycosides are not typical structurally of other CADs that induce phospholipidosis, and the kidney is not a usual phospholipidosis target tissue. Consequently, a possible association between aminoglycoside-induced phospholipidosis and related toxicity may not be applicable to nonaminoglycoside CADs.

Outside of the possible exception of aminoglycosides, there is no evidence that the presence of phospholipidosis is deleterious to the organism. While studies using phospholipidotic cells *ex vivo* can identify effects associated with the presence of the disorder in the cell, it may not be possible to extrapolate such effects to humans or to directly attribute them to the elevated phospholipids. The lack of adverse effects on cellular function under *ex vivo* conditions would suggest that toxicity should not develop *in vivo*. The primary advantage of *ex vivo* studies is the possibility of defining the influence of the lamellar bodies versus the free drug on cellular function. If it were possible to block the formation of lamellar bodies yet maintain intracellular CAD concentrations at the same level as when the bodies are present, it would enable the investigator to evaluate the role of the bodies in cellular toxicity. At present, there are no reports where that approach has been utilized.

Clearly, the most relevant way to approach this issue is to perform studies *in vivo*. Studies addressing the effect of phospholipidosis on tissue function using a biomarker to evaluate damage to the tissue would be helpful. For example, in animals, it is easy to monitor the effects of chemicals on liver function by measuring the levels of albumin or liver-specific enzymes in the blood in association with ultrastructural studies directly on the liver tissue. The absence of changes in liver function during the presence of phospholipidosis in the tissue would be evidence that the phospholipidosis is a toxicologically benign process under that circumstance. Alternatively, if an elevation in markers of liver injury would occur in association with a tissue, it would not be possible to determine if the damage was related to the inclusion bodies or a direct effect of the CAD on functions at another cellular site.

Over the past 30 years, great progress has been made in characterizing CAD-induced phospholipidosis ultrastructurally and biochemically. Unfortunately, the same cannot be said concerning the functional consequences of this condition. Efforts should be directed at designing experiments to definitively address this issue.

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