MINIREVIEW

Perspectives on Intracellular Storage and Transport of Cationic-Lipophilic Drugs (43584)

ZADOK RUBEN,*[,1] KURT J. RORIG,[†] AND SAM KACEW[‡]

Department of Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, New Jersey 07110; Chemo-Delphi Inc.,[†] Glenview, Illinois 60025; and Department of Pharmacology,[‡] University of Ottawa, Ottawa, Canada K1H8M5*

The cytoplasmic acidic vesicular compartment, which includes, in addition to lysosomes, endocytic, transport, Golgi, and secretory vesicles, plays an important role in the dynamics of cellular homeostasis in physiologic and disease states. This compartment is involved in processes of intracellular uptake, transport, digestion, synthesis, packaging, and storage as well as extracellular secretion. For example, the storage of lipid, apolipoproteins, and lytic enzymes in lamellar bodies under physiologic conditions serves an essential role in respiration. In particular, the lamellar bodies of lung epithelium are the site of storage for pulmonary surfactant. The lipid component of lung surfactant decreases surface tension to enable optimal gas exchange and a hydrophobic lining which provides protection against environmental influences (1). However, in certain pathologic conditions, as in atherosclerosis or in a number of inherited storage disorders, there is a massive accumulation of lipid-containing lamellar bodies (1–3). The inability of lysosomes to degrade this cellular material in lamellar bodies is proposed to be a factor in lysosomal storage disorders. Regardless of whether there is a deficiency in specific enzymes or transport carriers or an abnormal, excess substrate supply, the changes noted are a storage of uncatabolizable material primarily in nervous tissue, viscera, or both (4, 5). The clinical manifestations of each storage disease are dependent upon the distribution and severity of stored material. Over the last few years, a number of animal models of lysosomal storage disease have been developed for the elucidation of basic mechanisms of lysosomal functions.

The phenomenon of intralysosomal storage and lamellar body formation can be induced by numerous amphiphilic compounds and this has been the subject of several reviews (3, 6–8). Many of these amphiphilic agents are drugs previously classified as amphiphilic-cationic by Lüllmann *et al.* (9) based on the presence of lipophilic and cationic moieties, and are also known as cationic-amphiphilic or amphiphilic drugs. In this paper, the term cationic-lipophilic drugs (CLD) will be used to designate these amphiphilic compounds. It should be clearly noted that there is no direct relationship between the physicochemical nature of CLD and their pharmacologic properties or therapeutic uses (3, 6, 7). CLD have differing pharmacologic profiles and therapeutic uses, including antimalarial agents, antihistamines, antineoplastics, cardiac antiarrhythmics, and antidepressants.

Concomitant to the storage of lipid-protein-saccharide complexes in lysosomal lamellar bodies, lysosomes were also found to store CLD. Even though a great deal of research has been published on CLD and their storage in cells, the dynamic nature and the biologic meaning of storage is still poorly understood. In recent years, some light has been shed on various manifestations of storage, on the physicochemical properties of CLD responsible for storage in cells, on the involvement of the cytoplasmic acidic vesicular compartment in uptake, storage and transport of CLD, and on the toxicologic significance of storage. This information has provided newer perspectives on intracellular storage and transport of CLD and has been useful in discovery and design of new drugs. In this paper, we will review the biology of and provide perspectives on intracellular storage and transport of CLD.

[1] To whom requests for reprints should be addressed at Department of Toxicology and Pathology, Hoffmann-La Roche Inc., Building 100, 340 Kingsland Street, Nutley, NJ 07110-1199.

P.S.E.B.M. 1993, Vol 203: 140–149

0037-9727/93/2032-0140$3.00/0

Morphologic Manifestations of Storage of CLD

There are various morphologic manifestations of intracellular storage of CLD, the most common of which is concentric lamellar bodies. Electron microscopy is usually required for determining the morphologic features of the alterations associated with storage. The common feature to all alterations is distention of vesicles (membrane-bound vacuoles such as lysosomes and probably other cytoplasmic acidic vesicles). Prior to a description of the morphologic characteristics, the terminology used must be clarified. Synonyms given to the CLD-induced concentric lamellar bodies include myeloid bodies, myelinoid bodies, lamellated cytoplasmic inclusions, multilamellar inclusion bodies, lamellated bodies, lamellar bodies, and osmiophilic lamellar inclusions (7, 10). These are intravesicular, single-membrane, electron-dense concentric configurations. The membranous material is arranged in concentric layers with a periodicity of 40–50 Å (11) and was found to contain primarily stored phospholipid, which resulted from an inability of the cell to catabolize this substrate (12). The lamellae arise from the homogenous lysosomal matrix (3) and, as Reasor (13) demonstrated, may undergo further transformation into amorphous granular or membranous material. Terminology may be confusing and it is important to distinguish concentric lamellar bodies from cytoplasmic membranous whorls (or whorling bodies), which are a concentric proliferation of double membranes originating from either endoplasmic reticulum (e.g., the morphologic manifestation of microsomal enzyme induction associated with excessive treatment with phenobarbital) or from basal cell membrane (e.g., Mallory bodies associated with alcoholism).

Concentric lamellar bodies are believed to arise from heterophagic or autophagic processes. In autophagy, membranous material originates from within the cellular components such as organelles, endoplasmic reticulum, etc., and is surrounded by sequestering cisternae to form a vacuole. Subsequently, the thin membranes of sequestering cisternae are transformed into a thick limiting membrane of the lysosome. In the endoplasmic reticulum, the synthesized hydrolytic enzymes are transported within primary lysosomes to the autophagic vacuole. The sequestered organelles are digested by the hydrolytic enzymes and the autophagic vacuole is transformed into a heterogenous dense body or secondary lysosome. In heterophagy, the extracellular material enters the cell via endocytic/phagocytic vesicles; these fuse with primary lysosomes, which release hydrolytic enzymes and subsequently digest the captured material. Clearly, if for whatever reason the degradation of substrate sequestered within lysosomes is impaired, there is a resultant accumulation of substrate and the lysosome becomes a storage site (7).

Although the CLD-induced concentric lamellar bodies have been studied extensively, it should be noted that the presence of clear cytoplasmic vacuoles is another morphologic manifestation of CLD storage. These are distended membrane-bound vesicles whose content is predominantly electron-lucent material. Concentric lamellar bodies and clear cytoplasmic vacuoles may be recognized by histologic methods, and can be definitely differentiated by electron microscopic methods. In the toxicologic evaluation of the cardiac antiarrhythmic CLD disobutamide, Ruben *et al.* (14) found the predominance of clear cytoplasmic vacuoles over concentric lamellar bodies. Histochemical study of vacuolated cells revealed the presence of phospholipid accumulation (14) similar to that shown by Koizumi *et al.* (15). By electron microscopy, Ruben *et al.* (14) demonstrated that the disobutamide-induced changes in dog coronary artery muscle cells were in the Golgi area and included clear cytoplasmic vacuoles and concentric lamellar bodies. Most of these structures are membrane bound. The vacuoles become progressively larger with time and apparently accumulate electron-lucent material. Vacuolar size also increases by coalescence of adjacent vacuoles. There is morphologic evidence of vacuolar coalescence through a process of protrusion-invagination of adjacent vacuoles. Progressive vacuolar coalescence and accumulation of electron-lucent material results in cellular enlargement. It is of interest that other CLD, including tilorone, chloroquine, and piperamide maleate, were reported to induce the formation of clear cytoplasmic vacuoles (16–18).

Another morphologic feature associated with CLD storage is clear cytoplasmic vacuoles with electron-dense periphery; these have been linked with accumulation of glycosaminoglycans (19). Other morphologic features of storage are flocculent electron-dense structures as well as crystalline electron-dense bodies (3, 14). More importantly, combinations and variations of any of the morphologic alterations may occur. There is morphologic evidence for a relationship among the various electron-dense structures, the electron-lucent material, and the peripheral membrane of vesicles. It is possible that there are bidirectional processes between states of liquification and coacervation resulting in heterogeneity of the morphologic alterations. Artifactual effects caused by methods of tissue processing for microscopy should always be taken into account, and *in situ* observations on cultured cells may be advantageous.

Despite the importance of morphology, it is not possible to provide a pathogenomanic description for intracellular storage of CLD. There are a number of confounding factors to consider that complicate the issue. CLD may cause various alterations in different

animal species, in different cell types in an animal, and in different cell types in an organ. Similarly, in cultured cells, the predominant morphologic alterations induced by a CLD (e.g., tilorone) may vary dependent upon the duration of exposure and/or the concentration of the CLD (20, 21). More importantly, a correlation between the types of morphologic and chemical changes induced by CLD does not always exist.

Molecular Structure Determinants

The physicochemical characteristics of CLD associated with the development of concentric lamellar bodies have been reviewed extensively in recent years (8, 10). Physicochemically, the CLD molecule contains both a hydrophobic (lipophilic) moiety and a cationic hydrophilic moiety. The hydrophobic component of the molecule consists of a ring system that is usually aromatic but may contain both aromatic and aliphatic structures. The hydrophilic property resides in an amine moiety that is highly protonated at physiologic pH (22). Drugs with two basic amines and a well-defined lipophilic region, such as chloroquine and disobutamide, are capable of inducing a mixture of concentric lamellar bodies and clear cytoplasmic vacuoles (14, 17). For drugs with only one basic amine on the side chain and a well-defined lipophilic region, it was found that halogen substitution on the lipophilic ring structure, or on the basic amine side chain, resulted in a more potent induction of concentric lamellar bodies (23). The presence of a halogen on the aromatic ring of CLD, however, was not essential for induction of clear cytoplasmic vacuoles (24).

Extensive studies were carried out by Ruben and colleagues (14, 24–26) to establish structure-activity relationships of CLD and the induction of clear cytoplasmic vacuoles. The phenomenon was unraveled with the use of cultured cell preparations of either rabbit aorta muscle or rat urinary bladder carcinoma cells. The importance of the strongly basic cationic moiety as the determinant for the induction of clear cytoplasmic vacuoles was clearly demonstrated; a moiety of bis-tertiary amine, of which the pK_a of each amine is greater than 8.0, was found to be a highly potent vacuole inducer (24).

There is a complimentary link between the high basicity of the cationic moiety and the storage of CLD in the cytoplasmic acidic vesicular compartment; the vesicles of this compartment have three important properties pertaining to CLD storage: limiting membrane, acidic content, and distention capacity. In fact, disobutamide was found in the cell fraction of endocytic (and probably transport) vesicles as well as in the lysosomal fraction (27). CLD must cross membranes in order to move into or out of vesicles. The unprotonated state of the cationic moiety permits this movement, whereas the protonated state hinders it. CLD must also cross cell membranes in order to move into and out of cells; and, similarly, the protonated state of the cationic moiety would hinder cellular uptake, whereas the unprotonated state would permit it. This was shown by the pronounced effect of the pH of the culture medium: the more acidic (versus the more basic) medium decreased/delayed cellular uptake of disobutamide, and prevented/delayed appearances of vacuoles (25). In this study, there was parallelism between higher basicity of the culture medium, early appearance of vacuoles, and increased amounts of disobutamide in cells. Therefore, storage of disobutamide in cells is a combination of two factors: (i) the increased basicity of the culture medium provides more doubly unprotonated molecules, which can enter cells; and (ii) the acidic content of the cytoplasmic cellular acidic vesicles greatly diminishes the chances of doubly unprotonated molecules to occur, resulting in intracellular retention (storage) of this CLD. It has been proposed that the entrapped protonated amines cause an osmotic swelling of lysosomes, resulting morphologically in clear cytoplasmic vacuoles (28); this mechanism is a type of hydropic change.

The experimental model of varying the pH of the culture medium (14, 25) was also useful for demonstrating the importance of the highly basic bis-tertiary amine as a determinant in cellular uptake of CLD. Compared with the pronounced effect on uptake of disobutamide, there was only a slight effect on uptake of three cardiac antiarrhythmic CLD, structurally related to disobutamide except for having a cationic moiety of a monobasic amine (14).

By the use of cultured cells (as in whole animals), it was possible to demonstrate that not all CLD induce morphologic changes or store in cells (14, 24, 26). In fact, there are remarkable differences between very close structure analogs of CLD. The many advantages of cultured cells over whole animals for investigating the dynamics of the interactions of CLD with cells have been pointed out (3, 24, 29, 30). These include: (i) economy of time, test compound, and other research material; (ii) absence of effects due to poor absorption, first-pass metabolism, rapid elimination, or animal variability; and (iii) absence of artifactual effects due to tissue processing methods. The advantage of varying the pH of the culture medium (14, 23) is unique because the state of protonation of the cationic moiety is essential for passage of CLD through the cell membrane; achieving similar changes in extracellular pH would not be realistic in whole animals.

There was no difference in induction of clear cytoplasmic vacuoles by aliphatic diamines if the length of the aliphatic chain was two, four, or six carbons (24). In a recent study using 6-alkyl analogs of spectinomycin incubated with rat cultured hepatocytes, the length of the 6-alkyl side chain was found to be a factor in the

formation of concentric lamellar bodies and lysosomal storage, with the octyl derivative being more potent than the propyl compound (31). The reason for this difference may be attributed to increased lipophilicity of an eight-carbon chain.

The lipophilic moiety of CLD is important for induction of biochemical alterations associated with intracellular storage. This was shown in cultured cells (32) where a short aliphatic diamine of minimal lipophilicity induced clear cytoplasmic vacuoles not accompanied with significant alterations in saccharide and phospholipid contents, compared with the alterations in these substances induced by several CLD. These experiments also suggested that the lipophilic moiety plays a more important role in the induced biochemical alterations than just the anticipated elevation of intravesicular pH resulting from the entrapped protonated amines. The physicochemical properties of the lipophilic moiety which determine the biochemical alterations associated with CLD storage are not known.

When considering the role of the lipophilic moiety of CLD, it is important to note that there are compounds which possess a cationic moiety but are presumed to be amphiphilic. Aminoglycoside antibiotics such as gentamicin possess cationic groups; their ring structure, in contrast to CLD, is hydrophilic. These compounds may induce cellular alterations similar to those induced by CLD. Gentamicin produced a significant phospholipid accumulation in renal cortex particularly in the lysosomal fraction (33, 34). Based on these observations, it is our view that in addition to the cationic moiety, there is a specific structural requirement needed for the induction of biochemical alterations. In the case of gentamicin, the glycoside ring structure is hydrophilic, whereas in CLD the ring structure is lipophilic.

Phospholipid Alterations

The correlation between CLD-induced morphologic alterations, in particular the formation of concentric lamellar bodies and phospholipidosis, is well documented and has been the subject of several reviews (6, 35, 36). As shown in Table I, treatment with various CLD produces an increase in total phospholipid and in individual class phospholipids, including sphingomyelin, phosphatidylserine, phosphatidylinositol (PI), phosphatidylglycerol, phosphatidylethanolamine, and phosphatidylcholine as determined by chemical analysis. It is of interest that the CLD-induced elevation in individual-class phospholipid was not equivalent for all classes. In the case of chlorphentermine, the increase in lung phosphatidylcholine was highest (37), whereas PI was predominantly elevated in gentamicin-treated rat kidney (38, 39). Although desipramine did not appear to markedly affect total phospholipid, a significant rise was noted in rat lung and liver PI accompanied by a fall in phosphatidylethanolamine and sphingomyelin (40). It is of interest that in cochlear cells, it was found that gentamicin binding to PI prevented the hydrolysis of this lipid and the formation of inositol triphosphate (41, 42). Inositol triphosphate is believed to act as a second messenger for a calcium-dependent mobile response of outer ear hair cells. Apparently, binding of gentamicin to PI plays a role in the mechanism of ototoxicity.

Evidence of CLD induction of phospholipidosis has also been demonstrated histochemically with the use of various stains (7, 14). Various factors were found to influence CLD-induced phospholipidosis. Although the CLD share similar physicochemical characteristics, the induction of phospholipid accumulation is dependent upon tissue, species, and age (7). Subcellular localization studies revealed that the primary site for phospholipid accumulation is the lysosomal fraction (34, 43). The observed phospholipidosis appeared to be due to reduced catabolism as a consequence of binding of drug to phospholipid, which rendered the substrate less susceptible to phospholipases (12). Indeed, *in vivo* administration of gentamicin or chlorphentermine was found to inhibit phospholipases A and C in kidney and lung, respectively (44, 45). Similarly, incubation of various CLD with lysosomal preparations resulted in a marked inhibition in the activities of phospholipases A and C (12, 36). These findings suggest that impaired degradation of phospholipids is a common feature associated with CLD administration.

Glycosaminoglycan Alterations

The glycosaminoglycans (GAG), also termed mucopolysaccharides, contain derivatives of either glucosamine or galactosamine. The GAG are polyanionic substances of high molecular weight and consist of polysaccharide group side chains covalently linked to a polypeptide backbone. The significance of GAG in the pharmacologic action and toxicity of CLD is based primarily on the work of Lüllmann-Rauch and coworkers (Table II). As in the case of other CLD, tilorone was reported to induce the formation of lysosomal lamellar bodies and phospholipidosis as evidenced by morphologic and histochemical techniques, respectively (46); unlike most CLD, however, tilorone induced the formation of clear cytoplasmic vacuoles with electron-dense periphery. By means of cytochemical staining and radioactive uptake studies with ^{35}S, Lüllmann-Rauch (47) and Lüllman-Rauch and Ziegenhagen (48) demonstrated that the vacuoles were the sites of GAG storage and that these vacuoles were lysosomal organelles. With the use of fluorescence microscopy, the CLD were detected within the GAG-storing lysosomes (49). Based on the findings that the GAG levels induced by acridine derivatives were increased by a factor of five and 23 in liver and spleen,

Table I. An Example of CLD that Induce Phospholipid Accumulation as Determined by Chemical Assays

CLD	Tissue/cells assayed	Total phospholipid	Individual class phospholipid	Phosphotidylinositol	Ref.
Chlorphentermine	Lung	↑	↑	↑	37, 76
	Kidney		↑	↑	65
Diethylaminoethoxyhexestrol	Liver	↑	↑	↑	77, 78
Iprindole	Lung alveolar macrophages	↑	ND[a]	ND	79
Chlorcyclizine	Lung	↑	↑	↓	80, 81
	Lung	↑	↑	↑	82
Chlorimipramine	Lung	↑	↑	↑	83
Imipramine	Lung	↑	↑	ND	84, 85
Chloroquine	Lung	↑	↑	ND	84, 85
	Skeletal muscle	↑	ND	ND	86
	Cultured RAM[b] cells	↑	↑	↑	32
Amiodarone	Lung	↑	↑	↑	60, 87
	Cultured RAM cells	↑	↑	↑	32
Desipramine	Cultured fibroblasts	↑	↑	No change	61, 88
	Lung, liver	↑	↑	↑	40
Disobutamide	Cultured RAM cells	↑	↑	↑	32
Ambroxol	Lung	↑	↑	ND	89
Chloramitriptyline	Lung	↑	↑	↑	90

[a] ND, not done.
[b] RAM, rabbit aorta muscle.

Table II. Compounds that Induce Accumulation of Glycosaminoglycans in Lysosomes

Compound	Tissue/cells	Ref.
Tilorone	Liver	19, 91
	Tracheal chondrocytes/tibial cartilage	92, 93
	Cultured rat corneal, bovine, or human fibroblasts	20, 48
Tilorone analogs	Liver, kidney, spleen, cornea	46
Acridine orange and derivatives	Cultured corneal fibroblasts	21, 94
	Liver, spleen	49

respectively, Grave *et al.* (49) proposed that one drug molecule was bound to one disaccharide unit of GAG in the lysosome.

The precise role of GAG in CLD-induce storage, particularly in the accumulation of phospholipid, remains unclear. In a recent study, Ruben *et al.* (30) also found that tilorone induced the formation of clear cytoplasmic vacuoles in cultured rabbit aorta muscle cells. However, the observed morphologic change was not accompanied by phospholipid or mucopolysaccharide accumulation. It should be noted that in this study (30), the morphologic changes induced by disobutamide, chloroquine, and amiodarone were accompanied by increases in phospholipid and monosaccharide, but not mucopolysaccharide content. It has been demonstrated that GAG would prevent lipid deposition (50); it is, therefore, plausible that absence of GAG would be associated with elevated lipid levels.

It is known that there are differences in GAG content in different tissues and that lysosomal storage may be associated with a specific GAG. Tilorone produced a significant rise of dermatan sulphate but not of chondroitin sulfate or heparan sulphate in liver, kidney, and spleen (51). The cationic-lipophilic compound acridine, a more potent GAG-inducer, increased liver heparan sulfate and dermatan sulfate without a marked change in chondroitin sulfate (49). The differential effect on individual GAG has been also shown by lead, where in the presence of this metal, heparan sulfate was the major GAG affected (51). Thus, it is important to measure each individual GAG in order to establish a role of proteoglycans in intracellular storage of CLD.

It is of interest that gentamicin did not increase GAG in the cochlea but was found to bind to GAG (42). This binding was proposed to result in calcium mobilization and then inhibition of Na^+,K^+-ATPase (52). It is well established that gentamicin inhibits renal Na^+,K^+-ATPase and, perhaps, this aminoglycoside does bind to GAG (38). Evidence thus suggests that this aminoglycoside can induce an effect by binding to GAG without necessarily increasing the content of GAG or disturbing the function of lysosomal enzymes (1).

Saccharide Alterations, Glycosylphosphatidylinositol Anchors and Membrane Turnover

From morphologic observations on disobutamide-induced changes it was hypothesized that this CLD is associated with the processes of intracellular membrane turnover and transport (14, 53). Clear cytoplasmic vacuoles and concentric lamellar bodies were first seen in the Golgi vesicles of coronary artery muscle cells of dogs (14) and in the juxtanuclear area of cultured cells (25). By cell fractionation, the drug was found in fractions of endocytic and probably transport vesicles in addition to lysosomes (27). Furthermore, disappearance of vacuoles from cultured cells after withdrawal of disobutamide was associated with secretion of this CLD into the culture medium (54).

It is well known that proteins (e.g., enzymes) are attached to the membrane by covalent linkage with a glucosylphosphatidylinositol (GPI) moiety; the latter attaches to a nascent endoplasmic reticulum protein with the concomitant removal of a COOH-terminal (55, 56). Processing of the COOH terminal occurs on the luminal side of the endoplasmic reticulum and the mature GPI-anchored protein is subsequently transported to the plasma membrane. It is also well established that the GPI-anchored proteins are highly susceptible to cleavage by PI-specific phospholipases C and D (57, 58). However, a structural modification to the GPI-anchored proteins may render them resistant to the action of PI-specific phospholipases (57, 58).

Indeed, in a study (32), the CLD disobutamide, chloroquine, desipramine, and amiodarone produced in cultured rabbit aorta muscle cells a significant increase in PI and glucosyl residues, components of GPI anchors. This shows that storage of disobutamide may alter intracellular content of saccharide in addition to phospholipid. There is support for the association of CLD with membrane anchor molecules via findings on phospholipid, particularly PI (Table I). Martin *et al.* (59) found that incubation of cultured bovine pulmonary artery endothelial cells with amiodarone resulted in a significant rise in levels of PI and total phospholipid. In a recent study, Reasor *et al.* (60) reported that in alveolar macrophages of amiodarone-treated rats, there was a marked increase in content of total phospholipid and all individual classes of phospholipid. A rise in total phospholipid was produced by incubation of cultured human fibroblasts with desipramine (61), Madin-Darby canine kidney cells with chloroquine (62), and cultured rat peritoneal macrophages with chlorpromazine or amantadine (63, 64). It is of interest that the gentamicin-induced renal PI was also associated with a quantitatively higher percentage of increase in PI compared with other individual class phospholipids (33, 65). Furthermore, it should be noted that the gentamicin-induced nephrotoxicity is associated with inhibition of renal PI-specific phospholipase C (35, 66). It is thus conceivable that gentamicin increases the formation of GPI-anchored proteins, which cannot be degraded by phospholipases, and this is manifested as a resultant decrease in alkaline phosphatase (34, 65). The intracellular presence of gentamicin may also initiate a structural change in the GPI-anchored alkaline phosphatase or interfere with PI metabolism, which would be reflected as enzymic inhibition.

An increase in glucosyl residues and phosphatidylinositol content as described by Ruben *et al.* (32) is consistent with the suggestion that CLD may enhance membrane anchor synthesis. The mechanisms involved in the observed alterations in content of monosaccharides and phospholipid are not known; inhibition of lysosomal enzyme activities responsible for the degradation of phospholipid and membrane anchors could not be ruled out. Questions regarding the biologic effects of the potential for CLD to induce alterations in membrane anchors and contents of monosaccharides are open for research. Products of GPI anchor degradation may possess biologic activity and be involved in cell communications (67). Thus, alterations in the homeostasis of anchors may play a role in the mechanism of toxicity (42).

Several possibilities have been proposed for the mechanism by which CLD induces the alterations of storage of endogenous cellular substances and are reviewed by Kodavanti and Mehendale (8). Briefly, (i) drug binds to substrates (e.g., phospholipid), resulting in undegradable substrate-drug complex; and (ii) drug binds to enzymes (e.g., phospholipases), resulting in reduced degradation of substrates (3, 68, 69). A third possibility was proposed (32), that CLD bind to plasma membranes or intracellular membranes (e.g., of acidic vesicles, mitochondria, endoplasmic reticulum, and nucleus) with subsequent aberrations in membrane synthesis, recycling, turnover, and trafficking. Based on the wide variety of alterations in endogenous cellular substances induced by CLD (increases/decreases of phospholipid, GAG, monosaccharides, and possibly membrane anchors), the mechanism is not exclusive to one possibility; the wide variety of alterations suggests that multiplicity of specific physicochemical interactions may occur between CLD and cellular molecules. The wide variety of alterations further suggests that CLD interactions with enzymes and hydrophobic domains of other cellular molecules may be more important than the binding of CLD to substrate.

Investigations on Clear Cytoplasmic Vacuoles and Resultant Discovery of the Cardiac Antiarrhythmic Drug Bidisomide (SC-40230)

Design and discovery of new drugs may occur as a result of a deliberate process, based on scientific rationale stemming from investigating and elucidating of mechanisms of a biologic phenomenon. This was the

case in the investigations on clear cytoplasmic vacuoles induced by disobutamide and the discovery of its monobasic analog bidisomide.

At present, there is a need for new and more effective cardiac antiarrhythmic drugs to treat cardiac arrhythmias. Although various agents are currently available for the treatment of cardiac arrhythmias, the use of these drugs is limited by serious adverse effects or toxicities. Quinidine, the dextrostereoisomer of quinine, was serendipitously found to correct some cardiac arrhythmias; however, the compound possesses a low therapeutic index, with approximately one third of patients developing untoward effects including cardiotoxicity (70). Procainamide, an orally active derivative of procaine, was discovered to possess antiarrhythmic properties (71). Unfortunately, it has a short half-life of approximately 3 hr and is plagued by a high incidence of cardiotoxicity. Disopyramide, on the other hand, has a longer duration of action compared with the two previous drugs (70). Although it effectively suppresses atrial and ventricular arrhythmias, disopyramide causes marked anticholinergic side effects and reduces ventricular function. Disobutamide, the successor compound to disopyramide, was found to be an effective antiarrhythmic agent without producing significant adverse actions on the cholinergic system or ventricular muscle (72, 73). In the course of its preclinical safety evaluation, however, disobutamide was found to induce clear cytoplasmic vacuoles in various tissues of dogs and rats (14), which was a critical reason for withdrawing this CLD from further drug development. It should be noted that the presence of clear cytoplasmic vacuoles was not associated with other morphologic alterations indicative of cellular/tissue damage, nor with any overt functional impairment; presence of vacuoles merely reflected intracellular drug storage (14, 53).

A collaborative interdisciplinary research project was subsequently initiated to determine the biologic nature and the toxicologic significance of CLD-induced clear cytoplasmic vacuoles and the structural determinants of CLD that induce them. Within 24–72 hr after incubation with disobutamide, the induction of clear cytoplasmic vacuoles occurred in cultured cells of dog coronary artery muscle, rabbit aorta muscle, rat urinary bladder carcinoma, bovine aorta endothelium, Chinese hamster ovary tumor, and human skin and mouse fibroblasts (29, 30). The findings that the disobutamide-induced clear cytoplasmic vacuoles occurred in a wide range of cultured mammalian cells and that this phenomenon was equivalent to the *in vivo* condition (14, 15) clearly suggested that the use of cultured cells is an appropriate model for mechanistic research and extrapolation to the *in vivo* situation. Many observations were made by using cultured cells; the most pertinent to this topic are: (i) confirming the observations in whole animals that disobutamide-induced clear cytoplasmic vacuoles are a sign of storage of this CLD in cells; (ii) defining the role of the cationic moiety of CLD in induction of clear cytoplasmic vacuoles and delineating the physicochemical properties of this moiety for highly potent inducers; and (iii) confirming the observations made in whole animals that presence of disobutamide-induced clear cytoplasmic vacuoles is not associated with overt toxicity.

Based on the findings of the structure-activity relationships, bidisomide (SC-40230, compound 9[24]), a monobasic *N*-acetylated analog of disobutamide, was synthesized. Bidisomide is an effective cardiac antiarrhythmic and does not induce clear cytoplasmic vacuoles in cultured cells or in whole animals (14, 24). Currently, bidisomide is in phase II clinical trials, where it shows efficacy for ventricular and atrial arrhythmias.

Toxicologic Significance of Intracellular Storage of CLD

Toxicity implies functional impairment beyond physiologic limits. There is a need at times to define the borderlines of physiology in order to determine whether an induced change by a xenobiotic is a sign of toxicity. In the case of disobutamide, the induced clear cytoplasmic vacuoles in whole animals were a remarkable morphologic change that was not accompanied by necrosis, inflammation, atrophy, dysplasia, hyperplasia, metaplasia, or neoplasia (14); furthermore, presence of vacuoles was not accompanied by overt functional impairment of cells, tissues, or organs. Vacuolated cultured cells did not show a significant release of lactic dehydrogenase (20) or alterations in uptake of [^{3}H] thymidine (S. N. Anderson and Z. Ruben, unpublished observations). Other than vacuoles there were no ultrastructural alterations in cells whether cultured or in whole animals (14, 29). After drug withdrawal from whole animals or cultured cells, there was reversibility from a state of severe vacuolation (14, 54). It was concluded that presence of the induced vacuoles is a sign of intracellular storage, not of cellular degeneration (14, 53). Apparently, storage of this CLD in cells and the alterations it induces are within the physiologic limits of cells. On a similar situation for storage of chloroquine in the retina, Potts (74) stated: "It is clear that storage in pigment in itself is not a sufficient cause of toxicity."

Functional impairments including cell death, however, may be induced by CLD. The toxicologic significance of the induced observations of each drug should be determined separately. It is important, though, to determine the pathogenetic relationships of the toxic effects (functional impairment) to the induced morphologic changes (75); the latter may be nothing more than epiphenomena. Disobutamide induced cell death in cultured rat basophilic leukemia cells without the pres-

ence of cytoplasmic vacuoles (30); the mechanism of this cell death is not known.

Conclusions and Future Perspectives

The intracellular storage of CLD may manifest morphologically by various types of alterations. Combinations of these types may occur. Chemical alterations associated with storage are changes (primarily increases) in content of phospholipid, glycosaminoglycans and monosaccharides, and various combinations of these alterations may occur. There is no apparent correlation between the induced types of morphologic and chemical alterations. Storage of CLD is linked to the cytoplasmic acidic vesicular compartment, which includes endocytic, transport, Golgi, and secretory vesicles in addition to lysosomes. CLD-induced alterations in phospholipid and saccharide may play a role in intracellular storage and transport, especially with respect to cytoplasmic acidic vesicular trafficking and GPI anchors. The role of the cationic moiety in storage of CLD and the physicochemical features of this moiety for highly potent inducers of clear cytoplasmic vacuoles have been delineated. The lipophilic moiety is important for induction of biochemical alterations that accompany storage of CLD. The physicochemical properties of the lipophilic moiety, including the importance of the degree of lipophilicity, as determinant in induction of these biochemical alterations, remain to be elucidated. Not all CLD are stored in cells. Storage of CLD with its associated morphologic and biochemical alterations may or may not be a sign of toxicity. If CLD induce toxicity, this may be caused by other mechanisms not related directly to intracellular storage. Delineation of structure-activity relationships associated with storage enabled synthesis of new cardiac antiarrhythmic drugs. The association of CLD storage and absence of toxicity with intracellular transport and secretion is an extension beyond the concept of lysosomotropic agents (28). An interdisciplinary approach using a variety of investigative methods would be advantageous to further research on intracellular storage and transport of CLD; in this context, cultured cells are a highly advantageous research system. Further understanding of the biologic nature of the interactions of CLD with cellular processes could lead to a more lucid view on the toxicologic significance of cellular alterations induced by CLD, better understanding of the mechanisms of storage diseases, improved intracellular delivery of drugs, and the discovery of new drugs.

Note added in proof. Photomicrographs of CLD-induced morphologic changes are not included in this minireview. The photographic features of the morphologic changes are available in the cited original publications.

A major portion of this paper was written when Dr. Kacew was a visiting consultant at Hoffmann-La Roche Inc., Nutley, NJ. The generous support of Dr. Emil A. Pfitzer, Department of Toxicology and Pathology, Hoffmann-La Roche, Inc. is greatly appreciated. The assistance of Talora Stewart in typing the manuscript is acknowledged.

1. Schmitz G, Muller G. Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids. J Lipid Res **32**:1539–1570, 1991.
2. O'Brien JS. The gangiosides. In: Stanbury JB, Wyngaarden DS, Goldstein JL, Brown MS, Eds. The Metabolic Basis of Inherited Disease, 5th Ed. New York: McGraw-Hill, pp945–969, 1983.
3. Lüllmann-Rauch R. Drug-induced lysosomal storage disorders. In: Dingle JT, Jacques PJ, Shaw IH, Eds. Lysosomes in Applied Biology and Therapeutics. Amsterdam: Elsevier Science Publishers, pp49–130, 1979.
4. Murnane RD, Prieur DJ, Ahern-Rindell AJ, Parish SM, Collier LL. The lesions of an ovine lysosomal storage disease. Am J Pathol **134**:263–270, 1989.
5. Glew RH, Basu A, Prence EM, Remaley AT. Lysosomal storage disease. Lab Invest **53**:250–269, 1985.
6. Reasor MJ, Kacew S, Thoma-Laurie DL. Effects of cationic amphiphilic drugs on the developing animal. In: Kacew S, Reasor MJ, Eds. Toxicology and the Newborn. Amsterdam: Elsevier Science Publishers, pp69–84, 1984.
7. Hruban Z. Pulmonary and generalized lysosomal storage induced by amphiphilic drugs. Environ Health Perspect **55**:53–76, 1984.
8. Kodavanti UP, Mehendale HM. Cationic amphiphilic drugs and phospholipid storage disorder. Pharmacol Rev **42**:327–354, 1990.
9. Lüllmann H, Lüllmann-Rauch R, Wassermann O. Lipidosis induced by amphiphilic cationic drugs. Biochem Pharmacol **27**:1103–1108, 1978.
10. Reasor MJ. A review of the biology and toxicologic implications of the induction of lysosomal lamellar bodies by drugs. Toxicol Appl Pharmacol **97**:47–56, 1989.
11. Lüllmann H, Lüllmann-Rauch R, Wassermann O. Drug-induced phospholipidosis. Crit Rev Toxicol **4**:185–218, 1975.
12. Hostetler KY. Molecular studies of the induction of cellular phospholipidosis by cationic amphiphilic drugs. Fed Proc **43**:2582–2585, 1984.
13. Reasor MJ. Drug-induced lipidosis and the alveolar macrophage. Toxicology **20**:1–33, 1981.
14. Ruben Z, Dodd DC, Rorig KJ, Anderson SN. Disobutamide: A model agent for investigating intracellular drug storage. Toxicol Appl Pharmacol **97**:57–71, 1989.
15. Koizumi H, Watanabe M, Numata H, Sakai T, Morishita H. Species differences in vacuolation of the choroid plexus induced by the piperidine-ring drug disobutamide in the rat, dog and monkey. Toxicol Appl Pharmacol **84**:125–148, 1986.
16. Thelmo WL, Levine S. Renal lesions induced by tilorone and an analog. Am J Pathol **91**:355–358, 1978.
17. Frisch W, Lüllmann-Rauch R. Differential effects of chloroquine and of several other amphiphilic cationic drugs-upon rat choroid plexus. Acta Neuropathol **46**:203–208, 1979.
18. Benitz KR, Kramer AW. Piperamide-induced morphological changes in the choroid plexus. Food Cosmet Toxicol **6**:125–133, 1968.
19. Lüllmann-Rauch R. Tilorone-induced lysosomal storage mimicking the features of mucopolysaccharidosis and of lipidosis in rat liver. Virchows Arch [B] **44**:355–368, 1983.
20. Burmester J, Handrock K, Lüllmann-Rauch R. Cultured corneal fibroblasts as a model system for the demonstration of drug-induced mucopolysaccharidosis. Arch Toxicol **64**:291–298, 1990.
21. Lüllmann-Rauch R, Ziegenhagen M. Acridine orange, a precipitant for sulfated glycosaminoglycans, causes mucopolysaccharidosis in cultured fibroblasts. Histochemistry **95**:263–268, 1991.
22. Lüllmann H, Wehling M. The binding of drugs to different polar

lipids in vitro. Biochem Pharmacol **28**:3409–3415, 1979.
23. Joshi UM, Kodavanti PRS, Coudert B, Dwyer TM, Mehendale HM. Types of interaction of amphiphilic drugs with phospholipid vesicles. J Pharmacol Exp Ther **246**:150–157, 1988.
24. Rorig KJ, Ruben Z, Anderson SN. Structural determinants of cationic amphiphilic amines which induce clear cytoplasmic vacuoles in cultured cells. Proc Soc Exp Biol Med **184**:165–171, 1987.
25. Ruben Z, Anderson SN, Rorig KJ, Hribar JD, Bible RH Jr. The pH dependence of disobutamide-induced clear cytoplasmic vacuoles in cultured cells. Proc Soc Exp Biol Med **180**:84–91, 1985.
26. Anderson SN, Ruben Z, Gaud HT, Johnson RB, Gupta V. Induction of clear cytoplasmic vacuoles (CCV) in cultured cells by SC-35311 [Abstract]. Toxicologist **8**:86, 1988.
27. Hjelle JT, Ruben Z. Investigations in intracellular drug storage: Localization of disobutamide in lysosomal and nonlysosomal vesicles. Toxicol Appl Pharmacol **101**:70–82, 1989.
28. De Duve C, De Barsy T, Poole B, Trouet A, Tulkens P, Van Hoof F. Lysosomotropic agents. Biochem Pharmacol **32**:2495–2531, 1974.
29. Ruben Z, Fuller GC, Knodle SG. Disobutamide-induced cytoplasmic vacuoles in cultured dog coronary artery muscle cells. Arch Toxicol **55**:206–212, 1984.
30. Ruben Z, Anderson SN, Fuller GC. The susceptibility of various cultured cells to induction of clear cytoplasmic vacuoles by disobutamide. Toxicol In Vitro **4**:497–505, 1990.
31. Ulrich RG, Cramer CT. Potential to induce lamellar bodies and acute cytotoxicity of 6-alkyl analogues of spectinomycin in primary cultures of rat hepatocytes. Toxicol in Vitro **5**:239–245, 1991.
32. Ruben Z, Anderson SN, Kacew S. Changes in saccharide and phospholipid content associated with drug storage in cultured rabbit aorta muscle cells. Lab Invest **64**:574–584, 1991.
33. Feldman S, Wang MY, Kaloyanides GJ. Aminoglycosides induce a phospholipidosis in the rat renal cortex: An early manifestation of nephrotoxicity. J Pharmacol Exp Ther **220**:524–530, 1982.
34. Kacew S. Cationic amphiphilic drug-induced renal cortical lysosomal phospholipidosis: An in vivo comparative study with gentamicin and chlorphentermine. Toxicol Appl Pharmacol **91**:469–476, 1987.
35. Kacew S. Role of age in amphiphilic drug-induced pulmonary morphological and metabolic responses. Fed Proc **43**:2592–2596, 1984.
36. Reasor MJ, Kacew S. Amiodarone pulmonary toxicity: Morphologic and biochemical features. Proc Soc Exp Biol Med **196**:1–7, 1991.
37. Kacew S, Reasor MJ. Chlorphentermine-induced alterations in pulmonary phospholipid content in rats. Biochem Pharmacol **32**:2683–2688, 1983.
38. Kacew S, Bergeron MG. Pathogenic factors in aminoglycoside-induced nephrotoxicity. Toxicol Lett **51**:241–259, 1990.
39. Laurent G, Kishore BK, Tulkens PM. Aminoglycoside-induced renal phospholipidosis and nephrotoxicity. Biochem Pharmacol **40**:2383–2392, 1990.
40. Moor M, Honegger UE, Wiesmann UN. Organospecific, qualitative changes in the phospholipid composition of rats after chronic administration of the antidepressant drug desipramine. Biochem Pharmacol **37**:2035–2039, 1988.
41. Schacht J. Molecular mechanisms of drug-induced hearing loss. Hear Res **22**:297–304, 1986.
42. Grovaerts PG, Claes J, van De Heyning PH, Jorens PG, Marquet J, Broe ME. Aminoglycoside-induced ototoxicity. Toxicol Lett **52**:227–251, 1990.
43. Matsuzawa Y, Hostetler KY. Studies on drug-induced lipidosis: Subcellular localization of phospholipid and cholesterol in the liver of rats treated with chloroquine or 4,4′-bis(diethylaminoethoxy) a β-diethyldiphenylethane. J Lipid Res **21**:202–214, 1980.
44. Laurent G, Carlier MB, Rollmann B, Van Hoff F, Tulkens PM. Mechanism of aminoglycoside-induced lysosomal phospholipidosis: In vitro and in vivo studies with gentamicin and amikacin. Biochem Pharmacol **31**:3861–3870, 1982.
45. Kacew S. Role of phospholipase C in chlorphentermine-induced pulmonary phospholipidosis in rat. Proc Soc Exp Biol Med **188**:35–39, 1988.
46. Hein L, Lüllmann-Rauch R. Mucopolysaccharidosis and lipidosis in rats treated with tilorone analogues. Toxicology **58**:145–154, 1989.
47. Lüllmann-Rauch R. Lysosomal storage of sulfated glycosaminoglycans in renal interstitial cells of rats treated with tilorone. Cell Tissue Res **250**:641–648, 1987.
48. Lüllmann-Rauch R, Ziegenhagen M. Drug-induced lysosomal storage of sulfated glycosaminoglycans in cultured bovine and human fibroblasts. Virchows Arch [B] **60**:99–104, 1991.
49. Grave S, Lüllmann H, Lüllmann-Rauch R, Osterkamp G, Prokopek K. Induction of mucopolysaccharidosis in rats by treatment with immunostimulatory acridine derivatives. Toxicol Appl Pharmacol **114**:215–224, 1992.
50. Castellot JJ, Addonizio RD, Rosenberg RD, Karnovsky MJ. Cultured endothelial cells produce a heparin-like inhibitor of smooth muscle cell growth. J Cell Biol **90**:372–379, 1981.
51. Kaji T, Yamamoto C, Sakamoto M. Effect of lead on the glycosaminoglycan metabolism of bovine aortic endothelial cells in culture. Toxicology **68**:249–257, 1991.
52. Sitaras N, Vrouvidou P, Varonos D, Covas A. Metachromasia as the key to aminoglycoside ototoxicity: Preliminary report. Acta Otolaryngol **99**:336–338, 1985.
53. Ruben Z. The pathobiologic significance of intracellular drug storage: Clear cytoplasmic vacuoles. Hum Pathol **18**:1197–1198, 1987.
54. Ruben Z, Anderson SN, Hribar JD, Rorig KJ. Reversibility of disobutamide-induced clear cytoplasmic vacuoles in cultured cells following withdrawal of drug [Abstract]. Fed Proc **45**:574, 1986.
55. Major S, Menon AK, Cross GAM. Transfer of glycosyl-phosphatidylinositol membrane anchors to polypeptide acceptors in a cell-free system. J Cell Biol **114**:61–71, 1991.
56. Udenfriend S, Micanovic R, Kodukula K. Structural requirements of a nascent protein for processing to a PI-G anchored form: Studies in intact cells and cell-free systems. Cell Biol Int Rep **15**:739–759, 1991.
57. Low MG. Glycosyl-phosphatidylinositol: A versatile anchor for cell surface proteins. FASEB **3**:1600–1608, 1989.
58. Rosenberry TL. A chemical modification that makes glycoinositol phospholipids resistant to phospholipase C cleavage: Fatty acid acylation of inositol. Cell Biol Int Rep **15**:1133–1150, 1991.
59. Martin WG, Kachel DL, Vilen T, Natarajan V. Amiodarone pulmonary toxicity: Mechanism of phospholipidosis in cultured pulmonary endothelium cells. Clin Res **35**:535, 1987.
60. Reasor MJ, Ogle CL, Walker ER, Kacew S. Amiodarone-induced phospholipidosis in rat alveolar macrophages. Am Rev Respir Dis **137**:510–518, 1988.
61. Fauster R, Honegger U, Wiesmann U. Inhibition of phospholipid degradation and changes of the phospholipid-pattern by desipramine in cultured human fibroblasts. Biochem Pharmacol **32**:1737–1744, 1983.
62. Hostetler KY, Richman DD. Studies on the mechanism of phospholipid storage induced by amantadine and chloroquine in Madin Darby canine kidney cells. Biochem Pharmacol **31**:3795–3799, 1982.
63. Drenckhahn D, Kleine L, Lüllmann-Rauch R. Lysosomal alterations in cultured macrophages exposed to anorexigenic and psychotropic drugs. Lab Invest **35**:116–123, 1976.
64. Burmester J, Hanpft R, Kroplin K, Lüllmann-Rauch R, Pattern

M. Amantidine-induced lipidosis. A cytological and physicochemical study. Toxicology **44**:45–59, 1987.
65. Kacew S. Gentamicin or chlorphentermine induction of phospholipidosis in the developing organism: Role of tissue and species in manifestation of toxicity. J Pharmacol Exp Ther **232**:239–243, 1985.
66. Schwertz DW, Kreisberg JI, Venkatachalam MA. Effect of aminoglycosides on proximal tubule brush border membrane phosphatidylinositol-specific phospholipase C. J Pharmacol Exp Ther **231**:48–55, 1984.
67. Low M, Saltiel AR. Structural and functional roles of glycosylphosphatidylinositol in membranes. Science **239**:268–275, 1988.
68. Kubo M, Hostetler KY. Mechanism of cationic amphilic drug inhibitor of purified lysosomal phospholipase A. Biochemistry **24**:6515–6520, 1985.
69. Kubo M, Hostetler KY. Diethylaminoethoxyhexestrol inhibition of rat liver lysosomal phospholipase A_1 role of drug binding to substrate. J Pharmacol Exp Ther **240**:88–92, 1987.
70. Bigger JT Jr, Hoffmann BF. Antiarrhythmic drugs. In: Gilman AG, Goodman LS, Gilman A, Eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York: Macmillan Publishing, pp761–792, 1980.
71. Mark LC, Kayden HJ, Steele JM, Cooper JR, Berlin I, Rovenstine EA, Brodie BB. The physiological disposition and cardiac effects of procaine amide. J Pharmacol Exp Ther **102**:5–15, 1951.
72. Yonan PK, Novotney RL, Woo CM, Prodan KA, Hershenson FM. Synthesis and antiarrhythmic activity of α,α = bis[(dialkylamino)aklyl] phenylacetamides. J Med Chem **23**:1102–1108, 1980.
73. Dohrman ML, Harrell FE Sr, Strauss HC. The effects of disobutamide on electrophysiologic properties of canine Purkinje fibers and papillary muscle. J Pharmacol Exp Ther **217**:549–554, 1981.
74. Potts AM. Toxic responses of the eye. In: Amdur MO, Doull J, Klaassen CD, Eds. Casarett and Doull's Toxicology: The Basic Science of Poisons (4th ed). New York: Pergamon Press, pp521–562, 1991.
75. Ruben Z, Rousseaux CG. The limitations of toxicologic pathology. In: Haschek WM, Rousseaux CG, Eds. Handbook of Toxicologic Pathology. New York: Academic Press, pp131–142, 1991.
76. Schmien R, Seiler KU, Wassermann O. Drug-induced lipidosis 1. Lipid composition and chlorphentermine content of rat lung tissue and alveolar macrophages after chronic treatment. Naunyn Schmiedebergs Arch Pharmacol **283**:331–334, 1974.
77. De La Iglesia FA, Feuer G, McGuire EJ, Takada A. Morphological and biochemical changes in the liver of various species in experimental phospholipidosis after diethylaminoethoxyhexestrol treatment. Toxicol Appl Pharmacol **34**:28–44, 1974.
78. Tashiro Y, Watanabe Y, Enomoto Y. Experimental phospholipidosis induced by 4,4′-diethylaminoethoxyhexestrol. Acta Pathol Jpn **33**:929–942, 1983.
79. McNulty MJ, Reasor MJ. Iprindole-induced phospholipidosis in rat alveolar macrophages: Alterations in oxygen consumption and release of oxidants. Exp Lung Res **2**:57–69, 1981.
80. Kacew S. Alterations in newborn and adult rat lung morphology and phospholipid levels after chlorcyclizine or chlorphentermine treatment. Toxicol Appl Pharmacol **65**:100–108, 1982.
81. Reasor MJ, Heyneman CA, Walker ER. Chlorcyclizine-induced pulmonary phospholipidosis in rats. Res Commun Chem Pathol Pharmacol **38**:235–246, 1982.
82. Stern N, Teitz A, Gaton E, Wolman M. Effects of chlorocyclizine on pulmonary lipid metabolism in rats. Biochim Biophys Acta **754**:166–173, 1983.
83. Sgaragli GP, Corte LD, Gremigni D. Chlorimipramine-induced phospholipidosis: Biochemical and pharmacokinetic observations in the rat. Pharmacol Res Commun **15**:231–246, 1983.
84. Meerbach W, Grabner R, Muller U, Zimmermann W. Influence of imipramine and chloroquine on lung phospholipid content and lung structure in newborn rats. Exp Pathol **32**:225–232, 1987.
85. Grabner R, Meerbach W. Imipramine and chloroquine induced alterations in phospholipid content of rat lung. Exp Pathol **24**:253–259, 1983.
86. Nilsson O, Fredman P, Klinghardt GW, Dreyfus H, Svennerholm L. Chloroquine-induced accumulation of gangliosides and phospholipids in skeletal muscles. Quantitative determination and characterization of stored lipids. Eur J Biochem **116**:565–571, 1981.
87. Reasor MJ, Ogle CL, Kacew S. Amiodarone-induced pulmonary toxicity in rats: Biochemical and pharmacological characteristics. Toxicol Appl Pharmacol **97**:124–133, 1989.
88. Toplak H, Zuehlke R, Loidl S, Hermetter A, Honegger UE, Wiesmann UN. Single and multiple desipramine exposures of cultured cells. Changes in cellular anistotropy and in lipid composition of whole cells and of plasma membranes. Biochem Pharmacol **39**:1437–1443, 1990.
89. Wilke A, Muller B, Wichert P. Ambroxol increases the choline but not fatty acid incorporation into lung phospholipids in experimental lung disorders. Respiration **52**:129–136, 1987.
90. Karabelnik D, Zbinden G. Drug-induced foam cell reaction in rats II. Chemical analysis of lipids stored in lungs and foam cells after treatment with chlorphentermine, 5[p-(fluoren-9-ylidienemethyl)phenyl[-2-piperidineethanol] (RMI 10.393) and 1-chloramitriptyline. Hoppe-Seyler's Z Physiol Chem **356**:1151–1160, 1975.
91. Prokopek M. The tilorone-induced mucopolysaccharidosis in rats. Biochem Pharmacol **42**:2187–2191, 1991.
92. Lüllmann-Rauch R, Michel G, Peters A. Mucopolysaccharidosis-like cellular alterations in chrondrocytes of rats treated with tilorone. Exp Mol Pathol **49**:279–289, 1988.
93. Lüllmann-Rauch R, Peters A, Schleicher A. Osteopenia in rats with drug-induced mucopolysaccharidosis. Anzneim-Forsch **42**:559–566, 1992.
94. Handrock K, Laschke A, Lüllmann-Rauch R, Vogt RD, Ziegenhagen M. Lysosomal storage of sulfated glycosaminoglycosans in cultured fibroblasts exposed to immunostimulatory acridine derivatives. Toxicol Appl Pharmacol **114**:204–214, 1992.