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Minireview

Drug-induced phospholipidosis

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Abstract Drug-induced phospholipidosis is characterized by intracellular accumulation of phospholipids with lamellar bodies, most likely from an impaired phospholipid metabolism of the lysosome. Organs affected by phospholipidosis exhibit inflammatory reactions and histopathological changes. Despite significant advances in the understanding of drug-altered lipid metabolism, the relationship between impaired phospholipid metabolism and drug-induced toxicity remains enigmatic. Here we review molecular features of inheritable lysosomal storage disorders as a molecular mimicry of drug-induced phospholipidosis for an improved understanding of adverse drug reaction.

Keywords: Drug-induced phospholipidosis; Lysosome; Cationic amphiphilic drug; Lamellar body

1. Introduction

The appearance of microscopic subcellular structures induced by a variety of drugs led to recognition of a disorder that was subsequently termed drug-induced phospholipidosis (DIPL) [1]. Ultrastructural investigations revealed that these cytosolic inclusions consist of concentric myelin-like structures, the so-called lamellar bodies, the presence of which became the morphological hallmark of phospholipidosis [2]. Lamellar inclusion bodies are abundant in type II pneumocytes, where they function as storage vesicles and transporter of surfactant proteins. But in lysosomal storage disorders the lamellar bodies contain primarily undegraded phospholipids. Essentially, drug-induced phospholipidosis is characterized by phospholipid accumulation in affected tissue of which lung, liver, brain, kidney, cornea and others have been reported [3].

So far, more than 50 novel chemical entities have been identified to induce phospholipidosis. These include antibiotics, antidepressants, antipsychotics, antimalarial and antiarrythmic drugs. Many of these are cationic amphiphilic drugs (CADs) and share particular physical properties resulting from a chemical structure containing a hydrophilic ring and hydrophobic regions. Several of these drugs display severe adverse effects, such as acute pneumonitis or hepatitis as observed under amiodarone treatment [4]. However, the relationship between drug-induced phospholipidosis and adverse drug effects remains unexplained. Specifically, the onset of phospholipidosis may or may not be associated with clinical symptoms, including inflammatory reactions and histopathological changes, such as macrophagic infiltration or fibrosis. Clinically relevant phospholipidosis has been observed under administration of amiodarone, fluoxetine, gentamicin, perhexiline and 4.4′-dieethylaminoethoxyhexestrol [5].

The term drug-induced phospholipidosis ascribes to a condition that has been defined by the appearance of intracellular accumulation of phospholipids and lamellar bodies. Beyond this definition, each CAD induces its own specific phospholipid composition and selectively targets organs. There are species differences in drug-induced phospholipidosis and the presence of lamellar bodies may not always signify drug-induced phospholipidosis [6]. Animal models and cell culture systems have been used to study induction of lamellar bodies in response to several cationic amphiphilic drugs [7]. Essentially, the lysosome seems to be the primary target for drug-induced phospholipidosis and may serve as a storage organelle for phospholipid accumulation as well. Inhibition of lysosomal enzymes by CADs decreases phospholipid degradation thereby rendering lipid-drug particles indigestible [5]. As of today, drug-induced phospholipidosis remains an unresolved issue due to unclear molecular causes, and its sporadic relationship with adverse drug reactions. Indeed, the biological consequences of phospholipid accumulation in targeted organs such as liver or kidney remain, at least in part, speculative. Here we focus on drug-induced phospholipidosis, the origin of the lamellar bodies and its relationship to organ toxicity. Notably, some basic understanding on the various causes of phospholipidosis have been reviewed elsewhere [7,8].

2. The lamellar body

The lamellar bodies (also called: *lysosomal inclusion bodies* or *myeloid bodies*) are the pathologic–morphologic hallmark of phospholipidosis. In affected tissues, lamellar bodies can be seen by electron microscopy, where they frequently appear as intracellular concentric structures containing mainly deposits of undegraded lipids [6] (see also Fig. 1 and explanation herein). Normally, lamellar bodies serve as storage vesicle of surfactant in type II pneumocytes and were identified in pulmonary alveolar macrophages. These storage vesicles can be visualized by the Papanicolaou stain [9]. Lamellar bodies have also been

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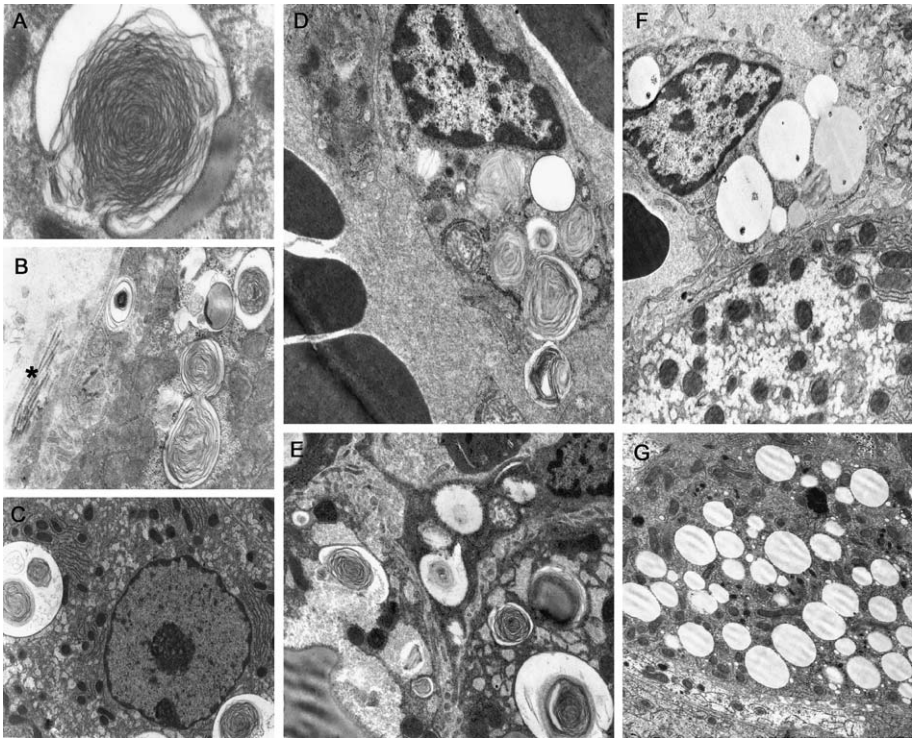

Fig. 1. (A) Lamellar body. (B) Fusion of two lamellar bodies inside the hepatocyte (∗ collagen fibres in the space of disse). (C) Hyperplasia of the endoplasmic reticulum during phospholipidosis. (D) Phagocytosis of lamellar bodies by Kupffer cells in the sinusoidal space. (E) Several lamellar bodies are located intracellular inside a hepatocyte that displays a high grade of vacuolization. (F) A Kupffer cell loaded with lipid droplets close to a disintegrated hepatocyte on the bottom of the picture. (G) In contrast, lipid droplets characterizing the steatosis.

observed in endothelium or cells of mesodermal origin such as gastric mucosa, in the peritoneum as well as pleural mesothelium [10]. In pathological conditions, however, these concentric bodies are typical for lysosomal storage disorders, such as the Niemann–Pick disease type I as well as drug-induced phospholipidosis. Furthermore, lamellar bodies are observed in cases of acute respiratory distress syndrome (ARDS) and in lecithin: cholesterol acyltransferase (LCAT) deficiency [11].

From the physiological context in which lamellar bodies arise, it is clear that they are somehow connected to biosynthesis and turnover of lipids. As mentioned above, lamellar bodies may serve as storage vesicles of surfactant or glucosylceramide, which is a precursor of ceramides and part of the epidermal permeability barrier. By topographic co-localization of lysosomal enzymes, the origin of lamellar body may be traced back to the lysosome. In addition, drug-induced lamellar bodies were shown to retain their fusion capabilities after drug withdrawal, which also supports a lysosomal origin [6].

Two principal hypotheses have been put forward to explain the formation of lamellar bodies, which typically consist of a lamellar core surrounded by a membrane (see Fig. 1). Indeed, Landmann described lamellar bodies as derived from membrane fission or budding of the trans-Golgi network to result in an unilamellar vesicle, that holds stacks of multiple flattened unilamellar vesicles (*lamellar body-disks*) [12]. This proposal has been criticized for its high energy requirement in the process of membrane fragmentation. In strong contrast, Norlén proposed a three-dimensional lipid structure model that consists of a separating outer cubic-like folded membrane with a larger lattice parameter and a single inner highly curved membrane, which corresponds to the lamellar structure of the inclusion bodies [13].

Despite uncertainties about origin and production of lamellar bodies and the varying circumstances in which they might appear, it is likely that lamellar bodies do not represent a homogenous entity; indeed, they display a broad spectrum amongst different species and organs. Specifically, their size varies between 100 and 2400 nm, they may appear uni- or multi-centred, contain apolipoproteins and have an acidic character, as observed in the lysosome [6]. Four functional types of lamellar bodies have been proposed by Schmitz and Müller deriving from either extracellular secretion (i), intracellular lipid storage (ii) extracellular origin, such as plasma lipid derived particles found in lecithin:cholesterol acetyltransferase (LCAT) deficiency (iii) or pathologic accumulation of extracellular membrane (iv). It is known that lamellar bodies may contain different phospholipids, their source, however, is still unidentified and may be generated during autophagic or heterophagic processes. Furthermore, strong evidence suggests involvement of the endoplasmic reticulum in the formation of lamellar bodies during autophagy [10].

Notably, cationic amphiphilic drugs were shown to bind phospholipids and to induce formation of aggregated phospholipid particles under certain circumstances [14].

Even though CADs were shown to induce phospholipidosis, the cationic amphiphilic moiety alone may not be the sole requirement for induction of multilamellar inclusions in DIPL.

For instance, the amphiphilic β-adrenergic drugs atenolol and sotalol and the local anesthetic and K^+ channel inhibitor procainamide showed no induction of lamellar body inclusion,

when compared to propanolol, which served as a positive control [15].

3. The lysosome in relation to drug-induced phospholipidosis

Even though phospholipidosis has been investigated extensively, no unifying mechanistic explanation has been put forward. Likely, the way by which amphiphilic drugs interact with the lysosomal system affects multiple pathways as detailed below.

The lysosome is part of the endosomal–lysosomal system, which was first discovered by Cristian De Duve in 1955 [16]. This cellular subcompartment is surrounded by a phospholipid bilayer and allows to maintain a v-type H^+-ATPase generated acidic milieu [17].

Approximately 40 different hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, proteases, phosphatases, sulfatases and several proteins of yet unknown function have been identified to composite the lysosomal content [18]. The hydrolyzing enzymes that have been contributed in form of proenzymes in vesicles of the endoplasmic reticulum (ER) and Golgi apparatus are activated by the low pH and are responsible for degradation of internalized micro- and macrophagic particles. These functions are termed endocytosis (heterophagy) or autophagy and describe connected lysosomal pathways in which either exogenous particles (such as cell debris, receptor-substrate complexes, etc.) or cytoplasmic constituents are affiliated and stored or recycled. The exocytotic activities of the lysosome play an important role in cell membrane repair and secretion of exogenous substances [19].

The lysosomal functions reach far beyond the recycling of cellular organelles and macromolecules. For instance, a group of lysosomal enzymes, the cathepsin proteases, play a crucial role in cell cycle control and antigen presentation. Their influence on cell motility, invasion and angiogenesis is mediated by degradation of extracellular matrix and has become a major focus of cancer-related research [20]. Lysosomal autophagy has been discussed as both an alternative non-apoptotic form of programmed cell death and a survival strategy during lean times. The role of autophagy and apoptosis in cell death and cell growth control needs to be further investigated, but the evidence available implies that alterations in the functioning of the lysosome may ascribe a significant participation during pathologic events [21].

4. CADs interact with phospholipids

Unlike hereditary lysosomal storage disorders, in which specific gene defects impair lysosomal function, drug-induced phospholipidosis is believed to result from several events caused by direct interaction of cationic amphiphilic drugs with phospholipids [8]. This assumption is based on two observations: Firstly, during phospholipidosis an accumulation of amphiphilic drugs in lysosomes is observed [5]. The strong affinity of drugs for the lysosomal compartment has been termed *lysosomotropic* behaviour [22]. Specifically, the affinity of cationic amphiphilic drugs can be explained by the acidic milieu of the lysosome and the weak basic properties of several xenobiotics. Chloroquine, for instance, is a weak base that is protonated after entering the low pH of the lysosome. The ionized form of the drug cannot pass through the hydrophobic core of the phospholipid bilayer that surrounds the lysosomal compartment and is therefore trapped [7]. Furthermore, the basic moieties of CADs raise the lysosomal pH, which coincides with phospholipidosis, but this effect occurs only temporarily and therefore may not represent a mechanism in the development of phospholipidosis [23]. Note, some CADs accumulate by lysosomal entrapment to result in high intralysosomal drug concentrations. In the case of treatment with aminoglycosides increases in lysosomal permeability were observed and coincided with activation of caspase-3 in kidney displaying phospholipidosis [24]. Next to diffusion, active uptake mediated by glycoreceptors into the lysosome has been proposed as a molecular avenue for lysosomal entrapment. Apparently, gentamicin-induced damage in the renal proximal tube is mediated specifically by megalin and when bound by this antibiotic the fusion of endosomes was inhibited [25]. It was demonstrated that uptake of cationic amphiphilic drugs into the lysosome correlates with therapeutic plasma concentrations [26,27]. Furthermore, even at low doses certain CADs can cause lysosomal alterations in cultured fibroblasts [28]. Redistribution of CADs after vitamin E administration is a mechanism believed to be responsible for the reversing effect of alpha-Tocopherol (alpha-Toc) on phospholipidosis [29].

Secondly, drug-induced phospholipidosis may arise from direct interaction of amphiphilic drugs with phospholipids [5]. For instance, chlorpromazine interacts with phospholipid membranes in a non-specific way, that increases membrane fluidity and enhances the release of neurotransmitters, thereby originating its therapeutic effect [30]. On the other hand, amiodarone positions in the hydrophobic core of the lipid bilayer where it alters the lipid dynamics. These interactions are believed to affect ion transport and oxidative phosphorylation that have been observed under amiodarone treatment [5]. The interactions of amphiphilic drugs and phospholipid bilayers are not homogenous [31]. A comparison of binding characteristics of several CADs to phosphatidylcholine revealed major differences. Indeed, while amiodarone was bound to the hydrophobic part, chlorphentermine and gentamicin displayed a strong affinity for the hydrophilic part, whereas chloroquine did not bind to either site of the phospholipid molecule [5]. The potency of an amphiphilic drug to induce phospholipidosis has been reported to correlate positively with the strength of its drug/phospholipid interactions [32,33]. Alterations in pH, pK_a, ion charge and hydrophobic properties of the lipid bilayer or partition coefficient may induce changes in the interaction between the drug and the phospholipid [5] and are therefore drivers of lysosomal entrapment of CADs [26].

5. Molecular mechanisms of drug-induced phospholipidosis

Basically two hypotheses have been put forward to explain the underlying mechanisms of drug-induced phospholipidosis [8]. The first hypothesis assumes that CADs bind directly to phospholipids to result in indigestible drug–lipid complexes, which accumulate and are stored in the form of lysosomal lamellar bodies [7]. The second hypothesis is based on the observation that production of lamellar bodies was associated with inhibition of phospholipase activity; either due to direct

inhibition or interaction of CAD at the phospholipid bilayer of the lysosome [8].

Lysosomal phospholipases play a critical role in the degradation of cellular membranes [34]. Specifically, phospholipase A1 (PLA1), Ca^{2+} dependent and independent phospholipase A2, and calcium-dependent phospholipase C were shown to be inhibited in a dose-dependent fashion by chlorpromazine, amiodarone and chloroquine. Furthermore, chlorpromazine, propanolol and chloroquine were found to mediate inhibition of phospholipase C activity by direct binding to the enzyme. The affinity for phospholipase A2 and its competitive inhibition has been variable among different amphiphilic substances [5]. Other lysosomal enzyme activities have been modulated by amphiphilic drugs, notably activity of the cathepsin B protease [35]. Enhanced activities of the lysosomal hexosaminidase, acid sphingomyelinase and sulfatase B in cells of the kidney cortex are considered to be compensatory increases of degrading enzymes due to phospholipase inhibition [36]. Interestingly, decreased sphingomyelinase activity was reported for cells of the renal cortex after gentamicin application [37]. Furthermore, chlorphentermine and gentamicin were shown to inhibit phospholiase C. While gentamicin inhibited the phosphatidylcholine and phosphatidylserine specific variants of phospholipase C, chlorphentermine inhibited a phosphatidylinositol specific phospholipase C [38].

Another possible mechanism for interference of CADs with phospholipase activity was proposed by Mingeot-Leclercq and co-workers, who had raised the *negative charge hypothesis*, by which phospholipase A1 inhibition resulted from charge neutralization of the lysosomal phospholipid bilayer [39,40]. This assumption was supported by studies with the erythromycin A derivative, azithromycin and the aminoglycoside gentamicin [39,41].

Recently, Piret et al. [42] reported that the activity of phospholipase A_1 is, in fact, influenced by the charged contents of the phospholipid bilayer. Increasing concentrations of negatively-charged lipids markedly enhanced activity of the phospholipase *A*. These findings are in support of the neutral charge hypothesis. Furthermore, secondary inhibition of phospholipase A1 could result from the accumulation of lysosomal phospholipids that influence the charge of the lysosomal bilayer in favour of neutral lipids.

It is of considerable importance that phospholipidosis is reversible to a certain point after withdrawal of the drug as demonstrated successfully *in vitro*, *in vivo* and clinically [43,44]. There are cases of phospholipidosis, however, where inhibition of lysosomal phospholipase A2 or sphingomyelinase persists [45]. Take note, that phospholipidosis was shown to occur within hours in cell culture experiments or after several months of treatment. Furthermore, the severity and organ/tissue specificity of phospholipidosis differed amongst drugs, but correlated with increased drug plasma concentrations. Likewise, the composition of accumulated phospholipids in phospholipidosis differ amongst drugs [5].

6. Phospholipidosis – a defence mechanism of the cell?

The biological consequence of phospholipid accumulations in several organs has not been evaluated to a point where lysosomal phospholipid storage and consecutive lysosomal dysfunction could clearly be linked to cellular toxicity. However, *in vivo* and *in vitro* investigations have given insights by which phospholipidosis does affect cellular function. After all, lysosomal protein degradation, fusion abilities, pino- and endocytosis have been impaired after CAD administration [8]. Furthermore, alveolar macrophages have the ability to scavenge lamellar bodies, which when completely packed become *foamy macrophages* during drug-induced phospholipidosis of the lung. Pulmonary macrophages have been studied intensively for functional consequences of phospholipidosis associated with the presence of lamellar bodies [46]. Enhanced phagocytic activity in pulmonary macrophages together with an increased production of bactericidal oxygen radicals might imply a shift of the immune response towards an enhanced unspecific cellular (phagocytic) response [47]. Indeed, the humoral immune response and in particular the delayed hypersensitivity response, the ability to activate antibody-secreting cells and the blastogenic response were significantly depressed after chlorphentermine administration in animal models [48]. This shift might suggest a defence mechanism of the cell when confronted with xenobiotics and their metabolites which otherwise would compromise the integrity of the cell.

Therefore, phospholipidosis might also be a part of a novel detoxification mechanism to prevent the cell from amphiphilic xenobiotics. Herein, the central role of the lysosome could lie in an activation of a cell survival strategy based on autophagy [21].

Lamellar bodies induced by phospholipidosis, have been observed to undergo exocytosis and have been located in the extracellular space. The images obtained by the electron microscopy are depicted in Fig. 1 demonstrate a hepatocyte releasing a lamellar body via exocytosis into the hepatic sinusoid during xenobiotic-induced phospholipidosis.

Notably, the Figure depicts phospholipidosis upon treatment of rats with Aroclor 1254, a mixture of polychlorinated biphenyls. This treatment resulted in steatosis, hyperplasia of endoplasmic reticulum and formation of lamellar bodies. Furthermore, scavenging of lipid droplets and lamellar bodies by Kupffer cells is documented.

Indeed, due to entrapment, CADs exert their major impact inside the lysosomal compartment. As long as the drug is confined to the lysosome, cells may be protected from toxicity. If so, inhibition of lysosomal enzyme activity could be a secondary effect. Thus, phospholipidosis could be part of a defence mechanism by which phospholipid recycle stacks accumulate, which enharbour a toxic xenobiotic [49]. By releasing the lamellar bodies via exocytosis, the cell bypasses excessive oxidation, detoxification and production of ROS (reactive oxygen species) thereby preventing undue stress. Cells of the macrophage system, such as the pulmonary macrophages and the Kupffer cells of the liver, scavenge the lamellar bodies and facilitate their removal for further clearance of the lamellar body/xenobiotic complex. Enhanced lysosomal enzyme release and a dose-dependent clearance of CADs into the bile may be suggestive for an enhanced exocytotic activity [50]. It is probable, that such mechanism is limited to certain drugs and may be saturable. Once a drug exceeds certain concentrations, lysosomal permeability is a likely event [51]. For instance, apoptosis, mitochondrial swelling and lactate dehydrogenase (LDH)-release during phospholipidosis has been reported for the proximal renal tubular cells upon treatment with aminoglycosides [24].

Take note, that aminoglycosides cause acute renal toxicity due to tubular necrosis. Several factors have been suggested

to be responsible for this, such as mitochondrial enzyme inhibition and peroxidation of phospholipids [24]. Furthermore, secondary effects of increased cellular phospholipid, e.g., inhibition of protein synthesis, inhibition of phospholipase C as well as an impairment of protein kinase C have been discussed [8]. Indeed, neither phospholipase inhibition nor phospholipid accumulation alone resulted in cell toxicity, but the nephroprotective effect of polyaspartic acid administration had brought phospholipidosis back to the centre of discussion. Polyaspartic acid is transported to the lysosome via endocytosis and builds complexes with the aminoglycosides gentamicin or amikacin. In such complexes aminoglycosides were stored in a non-toxic form and prevented interaction with phospholipids [24]. Aminoglycosides display a broad range in their potency to induce apoptosis and interference with the mitochondrial electron transport [24]. The role of phospholipidosis in this toxicity remains speculative [52]. As of today, only a few cases of toxicity induced by cationic amphiphilic drugs could be directly linked to phospholipidosis [8], but the β-blocker propranolol and the Ca^{2+}-antagonist verapamil were shown to decrease mitochondrial volume density associated with a significant dose-dependent LDH-release in hepatocytes at subtoxic concentrations [15]. Recently, amiodarone and imipramine were shown to inhibit cell proliferation and to activate caspase-mediated apoptosis in cell culture [53].

7. Learning from lysosomal storage diseases for an improved understanding of drug-induced phospholipidosis

Lysosomal dysfunctions have been studied in various heritable lysosomal storage diseases including mucopolysaccharidoses, sphingolipidoses, e.g., the Gaucher's disease, and others, namely the Niemann–Pick type C disease. It is of considerable importance that the disease phenotypes are heterogeneous, afflict multiple organ systems and are always progressive. A variety of lysosomal defects thus lead to high accumulations of lysosomal substrates. For the majority of lysosomal storage disorders (LSD), the exact pathophysiological mechanisms remain unclear. Several mutations have been identified in the open reading frames of genes coding for lysosomal hydrolases to affect synthesis, folding, activation or targeting of substrates. Furthermore, membrane protein defects and impaired transport processes have been observed.

The classic lysosomal storage disorder is monogenic and causes accumulation of lysosomal substrate due to defective enzymes or proteins. A defective lysosomal hydrolase, for instance, is found in the neurogenerative Tay-Sachs disease, where impaired function of the β-hexaminidase A leads to accumulation of GM2 gangliosides and glycolipids.

Once again, mutations in genes that encode for enzymes can lead to a variety of deficiencies, including undue post translational modifications. This has been observed in different multiple sulphatase deficiencies (MSD) [54].

In Fabry's disease, deficiency of the galactosidase A results in accumulation of globostriasyl-ceramide. Interestingly, a recent report compared the pathology of chloroquine-induced phospholipidosis and the Fabry's disease. The anti-malarial and anti-rheumatic drug chloroquine is a potent inhibitor of the lysosomal phospholipase A and C, believed to be responsible for impaired phospholipid degradation and accumulation. Note, chloroquine was also shown to reduce activity of the α-galactosidase A. Both Fabry's disease and chloroquine-induced phospholipidosis induced a similar pattern of inclusion bodies, specifically, in renal cells, endothelial cells and striated myocardial muscle cells. Furthermore, in patients of Fabry's disease and in chloroquine-induced phospholipidosis the clinical manifestations were similar, e.g., decreased renal function associated with glomerular proteinuria and edema, which can be explained by secondary effects of excessive lysosomal storage and sensitivity to α-galactosidase A deficiency [55].

Despite different genetic causes the subcellular phenotypes of inheritable lysosomal storage diseases are similar and frequently due to substrate overload. Furthermore, it was hypothesized that accumulation of one lipid *raft* would lead to trapping of other lipids within the lysosome [57]. For instance, the Niemann–Pick disease type C (NPC) results from a defect of cholesterol trafficking due to a defective NPC-1 protein that is involved in cholesterol transport from the late endosomes and lysosomes to other organelles. In patients diagnosed with NPC, a wide range of neurological symptoms can be observed. These symptoms, seem however not to arise from accumulation of cholesterol, but from accumulation of glycosphingolipids (GM2) [58]. Therefore, despite impaired cholesterol transport in Niemann–Pick disease, type C sphingolipid accumulation occurs as well. Likewise in Niemann–Pick disease type A, a defect of the sphingomyelinase leads to accumulation of sphingomyelin that is accompanied by accumulation of cholesterol. It is of considerable importance that in drug-induced phospholipidosis, accumulation of phospholipids in lamellar bodies is also accompanied by increased levels of neutral lipids and cholesterol. [59] In addition, second raft accumulation is also expected to alter the membranes of the endosomal/lysosomal system and to contribute to the formation of lamellar bodies in lysosomal storage diseases by flattening the highly curved internal membrane [57].

Changes of the cellular cholesterol turnover are sensored by the sterol regulatory element binding proteins (SREBP) type transcription factors and posttranslational by proteins that contain a sterol sensing domain (SSD), such as the NPC-1 protein and the HMG-CoA reductase [60]. Specifically, the SREBPs regulate multiple genes of the cholesterol biosynthesis and uptake pathway, including the HMG-CoA reductase and HMG-CoA synthase [61]. It is of considerable interest that in a recent toxicogenomic study the majority of drugs tested for their potency to induce phospholipidosis provoked transcriptional upregulation of HMG-CoA reductase and HMG-CoA synthase [62].

Furthermore, the HMG-CoA reductase is embedded in the membrane of the endoplasmic reticulum and was shown to increase intracellular cholesterol, which triggered the unfolded protein response in macrophages [56]. Likely, lipid overload of the lysosome contributed to the advancement of drug-induced phospholipidosis.

The genomic response to phospholipidosis includes attenuation of transcription of several genes coding for lipid metabolism and transport, such as the ELOVL (elongation of long chain fatty acids) family member 6 and the FABP (fatty acid binding protein) [62].

An impairment of lysosomal lipid transport has also been suggested to be involved in the pathology of NPC and in the formation of lamellar bodies in respiratory distress syndrome (RDS). The ATP-binding cassette (ABC) transporter A1 (ABCA1) ABCA1 mediates the rate-limiting step in high

density lipoprotein (HDL) particle formation of the liver and is regulated by cellular cholesterol. Impairment of this enzyme was shown to be responsible for an impaired lipid efflux in Niemann–Pick disease type C [63]. Formation of lamellar bodies in fatal respiratory distress syndrome (RDS) in the newborn has been linked to mutations of the ABCA3. The ABCA3 gene is exclusively expressed in the lung, specifically in type II cells and responsible for transport of surfactant protein A. Knockdown of endogenous ABCA3 caused formation of abnormal lamellar bodies, while the vesicular uptake of phosphatidylcholine, sphingomyelin and cholesterol but not phosphatidylethanolamine was decreased [64].

Besides, the distinct pathology of some lysosomal storage diseases result from decreasing lysosomal stability, as suggested elsewhere [54]. Indeed, accumulation of undegraded lipid substrates might cause instability or rupture of the lysosomal membrane above a certain threshold and thus results in leakage of lysosomal hydrolases into the cytoplasm [54].

The involvement of lysosomal proteases in apoptotic processes has been described earlier in this review. Indeed, apoptosis of renal cells due to aminoglycosides was shown to be associated with decreasing lysosomal stability and leakage [52], where as chlorphentermine was shown to stabilize the lysosomal membrane in alveolar macrophages during animal studies [65]. Therefore, drugs differ in their ability to interfere with the lysosomal membrane.

An investigation of hereditary lysosomal storage disorders revealed the importance of secondary effects due to accumulation of material within the lysosome for the actual pathology of the phenotype [66]. For instance, inflammatory response and macrophages activation was shown to be responsible for the major neuronal damage in Sandhoff's disease, a lysosomal storage disorder characterized by storage of gangliosides in the central nervous system [67]. Macrophage activation is observed in many lysosomal storage disorders and also in pulmonary drug-induced phospholipidosis. In Gaucher's disease, for instance, in which lysosomal glycolipid storage occurs, macrophage infiltration was reported in association with proteinuria [68]. Recently, the presence of prominent histiocytes in association with lysosomal inclusions in glomerular capillary lumina have been described as distinct features of chloroquine-induced renal damage [55]. Next to lysosomal substrate accumulation, impaired mitochondrial function was observed in neuronal ceroid lipofuscinosis and in cells from patients with Fabry's disease [66]. Correspondingly, a loss of mitochondrial function accompanied by loss of cell viability was detected in pulmonary phospholipidosis induced by chlorphentermine and amiodarone [69,70].

In summary, secondary effects of increased lysosomal storage found in both LSD and DIPL, include changes of lipid synthesis and trafficking, macrophage activation and impaired mitochondrial function. Even though the pathophysiology of lysosomal storage disease has not been completely understood, the study of distinct phenotypes may help to gain mechanistic insight into drug-induced phospholipidosis.

8. Conclusion

Drug-induced phospholipidosis presents an unresolved condition that occurs frequently with a wide range of drugs. Even though much effort has been spent to investigate the molecular effects and functional consequences of drug-induced phospholipidosis, the mechanistic link to adverse drug reactions is unresolved. Therefore, the causality of phospholipid accumulation, as well as the occurrence of lamellar bodies as a consequence for organelle dysfunction and organ toxicity, remain enigmatic. An improved understanding of phospholipidosis will result from genomic analysis, where several drugs known to cause phospholipidosis induced multiple gene expressions coding for proteins of the lysosomal integrity and function. Among them, 12 genes could be classified in four functional categories that are involved in downregulated phospholipase activity and lysosomal enzyme transport as well as upregulation of cholesterol and phospholipid synthesis [62].

It remains unclear whether phospholipidosis may arise from impaired lysosomal enzyme activities or formation of indigestible CAD–phospholipid complexes. Furthermore, altered lipid concentrations in phospholipidosis may originate from increased phospholipid synthesis, decreased degradation or impaired transport of phospholipids. All of these possibilities have been associated with phospholipidosis, but neither the sequence in which they appear, nor their molecular causes have been elucidated sufficiently, as yet.

Individual sensitivity may be a determining factor for phospholipidosis and a unifying hypothesis for phospholipidosis by interference with cationic amphiphilic drugs can not be put forward [7]. Emerging evidence is suggestive for phospholipidosis to protect cells from undue stress, at least in part, by drug removal and clearance of lamellar bodies which enharbour lipid–drug complexes [71]. Results from investigations into lysosomal storage diseases may direct future research in DIPL by focusing on lysosomal effects of phospholipid storage and, in particular, changes in gene expression, lysosomal signalling pathways and lipid metabolism as well as its trafficking. Likely, these studies will permit dissection of altered lipid metabolism, secondary effects of phospholipid accumulation and effects linked to adverse drug reactions [62,72].

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