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Drug induced phospholipidosis: An acquired lysosomal storage disorder ☆

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ABSTRACT

There is a strong association between lysosome enzyme deficiencies and monogenic disorders resulting in lysosomal storage disease. Of the more than 75 characterized lysosomal proteins, two thirds are directly linked to inherited diseases of metabolism. Only one lysosomal storage disease, Niemann–Pick disease, is associated with impaired phospholipid metabolism. However, other phospholipases are found in the lysosome but remain poorly characterized. A recent exception is lysosomal phospholipase A2 (group XV phospholipase A2). Although no inherited disorder of lysosomal phospholipid metabolism has yet been associated with a loss of function of this lipase, this enzyme may be a target for an acquired form of lysosomal storage, drug induced phospholipidosis. This article is part of a Special Issue entitled Phospholipids and Phospholipid Metabolism.

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1. Introduction

The discovery of the lysosome was reported by de Duve in 1955 [1]. The significance of de Duve's seminal work was immediately appreciated, and he shared the Nobel Prize in 1974. Almost 60 years later, the importance of this work remains unquestioned in no small measure due to the subsequent identification of over 50 inherited diseases resulting from the inherited loss of function of single gene products that localize to the lysosome [2]. The study of these disorders has been first and foremost valuable for the identification, management and treatment of these diseases which, although rare, collectively account for 1 in every 8000 births [3]. Of comparable importance have been the insights into the biochemistry and cellular biology of lysosomal substrates and the role of this organelle in fundamental cellular processes including autophagy, host defense, and cell death. For example, several metabolic pathways would not have been as readily identified and the focus of attention if not first identified through the characterization of these genetic diseases. Included in these pathways are those involving glycosphingolipids, mucopolysaccharides, and cholesterol esters. Insights derived from investigation of protein trafficking and vesicle sorting would not have been as readily pursued if not for de Duve's observations.

In contrast to studies on these inherited disorders, considerably less attention has been focused on the concept that there may be acquired forms of lysosomal storage and that in the study of such cases additional insight into cell metabolism and cellular biology might be gleaned. This review is based on the premise that there is at least one form of acquired lysosomal storage, viz. drug induced phospholipidosis. It is argued that an understanding of drug induced phospholipidosis is not only important to the field of drug discovery and development and toxicology, but that fundamental questions surrounding drug induced phospholipidosis might be more tractable if studied in the context of lysosomal biology. In support of this premise, this review will focus on the discovery and characterization of a newly identified lysosomal enzyme, group XV phospholipase A2 [4] and its potential role as a mediator of some forms of drug induced phospholipidosis.

2. Phospholipid metabolism in the lysosome

The recognition that the lysosome was an important site for the catabolism of phospholipids was appreciated with the recognition that inherited deficiencies in acidic sphingomyelinase are the basis for Niemann–Pick disease types A and B [5]. The choline phosphohydrolase activity of this enzyme against sphingomyelin results in the formation of ceramide and phosphorylcholine. Due to the clinical importance of Niemann–Pick disease, much of the published work on lysosomal phospholipid metabolism has focused on the catabolism of sphingomyelin and less attention on more abundant phospholipids such as phosphatidylcholine and phosphatidylethanolamine. However, additional hydrolytic activities against glycerophospholipids localized to cellular

Abbreviations: BMP, bis(monoacylglycero)phosphate; CAD, cationic amphiphilic drug; DIP, drug induced phospholipidosis; GXVPLA2, group XV phospholipase A2; LPLA2, lysosomal phospholipase A2; MDCK, Madin Darby canine kidney; PDMP, D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-propanol

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lysosomal fractions and characterized by acidic pH optima do exist and were in fact first reported 45 years ago. These enzyme activities include phospholipase A1, A2, C and D.

A phospholipase A activity obtained from rat liver lysosomes was first described by Mellors and Tappel in 1967 [6]. This calcium independent enzyme had an acidic pH optimum and recognized both phosphatidylcholine and phosphatidylethanolamine as substrates. Similar phospholipase A activities in rat liver were confirmed in other reports [7,8]. Subsequently, distinct phospholipase A1 (PLA1) and A2 (PLA2) activities were separated by gel filtration form the soluble fractions of rat liver lysosomes [9]. Additional sources of lysosomal phospholipase A activities were subsequently reported from sources that include myocardium [10], alveolar macrophages [11], arterial smooth muscle cells [12], testes [13], renal cortex [14], and peritoneal macrophages [15].

An acidic phospholipase C activity from homogenates of rat tissues including fat, brain, diaphragm, duodenum, heart, small intestine, kidney, liver, lung, skeletal muscle and spleen has also been identified [16]. In addition, lysosomal phospholipase C was identified from the soluble fractions of rat liver [17–19] and kidney [14] lysosomes, and canine cardiac sarcoplasmic reticulum [20]. The enzyme activity is characterized by has an acid pH optimum and the absence of any requirement for divalent metal ions. An uncharacterized phospholipase D activity may also be localized to lysosomes [21].

Several lysosomal PLA1s were purified from rat liver and kidney by Hostetler and colleagues. They isolated five lysosomal PLA1s with molecular weights between 20,000 and 90,000 from rat liver [22]. The differences in molecular weights between the isolated enzymes were due to distinct isoelectric points and carbohydrate moieties. Each enzyme demonstrated an acid optimum pH, the absence of a requirement of divalent metal ions, and a higher specificity toward *sn*-1 position compared to the *sn*-2 position of glycerophospholipids. The lysosomal PLA1 purified from rat kidney cortex was a glycoprotein with an isoelectric point of pH 5.4 and an apparent molecular weight of 30 kDa [23]. However, the lysosomal PLA1 gene and encoded amino acid sequence have not been reported. It should also be noted that a lysosomal-type Ca^{2+}-independent PLA2 was isolated from rat lungs and cloned from a human myeloblast cell line [24]. The characterized enzyme was identical to peroxiredoxin 6 and is a bi-functional enzyme with activities that include glutathione peroxidase and phospholipase A2. It has been suggested that when the enzyme is located in the cytoplasm it functions as a glutathione peroxidase and when in lysosomes as a phospholipase A2 [25].

At a cellular level, there are unique obstacles that must be overcome for a phospholipase to function within the lysosome. First, the lipid substrates are present within complex membranes and must be presented to the enzyme. Second, the limiting membrane around the lysosome, although protected by a glycocalyx, is itself a potential target of the hydrolytic enzymes within the lysosome. Thus in some manner there must be separation between lysosomal enzymes and the outer lysosomal membrane. Third, the products of phospholipid and sphingolipid metabolism, including sphingosine, ceramide and lyso-phospholipids, themselves are capable of permeabilizing the lysosomal membrane, a process linked to cell death. Thus there should be some mechanism by which the accumulation of these products is limited.

Sandhoff has suggested that there are key properties shared by lysosomal hydrolases, including acid ceramidases, sphingomyelinase, and lysosomal phospholipase A2 that address these challenges [26]. First, these enzymes function within a subcompartment in the lysosome, likely against intra-lysosomal vesicles, where they are prevented from contacting the limiting membrane. Second, these enzymes or their associated activating proteins bind to anionic phospholipids at the acidic pH found in the lysosome. Included among these anionic lipids is BMP, a phospholipid uniquely found only the lysosome and late endosome [27]. The lysosomal lipid binding proteins include saposins or ganglioside GM2 activating protein. Third, these enzymes run their reactions in both forward and reverse preventing the reaction products from accumulating at too great a concentration [28]. Fourth, the anionic lipids to which the hydrolase bind turn over very slowly (Fig. 1).

3. BMP a phospholipid uniquely found in the late endosome/lysosome

Bis(monoacylglycero)phosphate (BMP) is an anionic phospholipid uniquely found in lysosomes and late endosome [29]. BMP has a stereoconfirmation that renders it a poor substrate for catabolism by lysosomes. BMP is negatively charged at lysosomal pH. Finally, BMP is localized to the inner membranes of lysosomes and late endosomes. BMP therefore is considered the primary anionic phospholipid that binds lysosomal hydrolases. BMP essentially consists of two monoacylglycerols bound to a single phosphate. For this reason the commonly used name for this lipid, lyso-bisphosphatidic acid, is incorrect. The resistance of BMP to phospholipases is most probably due to its *sn*-1:*sn*-1′ configuration. Although some studies have suggested that this structure consists of a phospholipid with fatty acyl groups esterified groups at the *sn*-2:*sn*-2′ positions. The fatty acid chains vary in composition and include oleic acid and polyunsaturated fatty acids.

The site of synthesis of BMP within cells has not been established although it may occur at the inner membrane surfaces of late endosomes and lysosomes. Given that 10% of BMP is in the late endosome and 90% is in the lysosome, it is reasonable to assume that synthesis occurs in one or both of these sites [30]. If this is true,

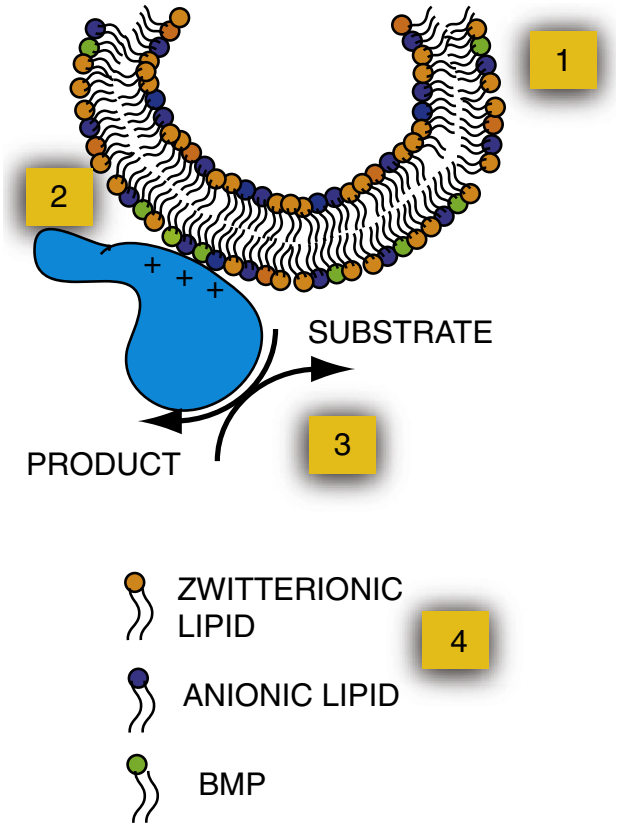

Fig. 1. Common properties of lysosomal hydrolases. (1) There is subcompartmentalization leading to separation from the limiting membrane. The hydrolases are active against intralysosomal vesicles that contain the substrates for these enzymes. (2) A binding interaction occurs between the enzyme and the intralysosomal membrane at acidic pH. (3) The hydrolases catalyze reactions reversibly. (4) The anionic phospholipids within the membranes such as BMP and phosphatidylinositol are slowly metabolized.