Biochemical and Biophysical Research Communications 377 (2008) 268–274

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Drug-induced phospholipidosis is caused by blockade of mannose 6-phosphate receptor-mediated targeting of lysosomal enzymes

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ARTICLE INFO

Article history:
Received 25 September 2008
Available online 7 October 2008

Keywords:
Drug-induced phospholipidosis
Cationic amphiphilic drug
Mannose 6-phosphate/IGF-II receptor
Lysosomal enzymes
Endosomes

ABSTRACT

Cationic amphiphilic drugs (CADs) cause massive intracellular accumulation of phospholipids, thereby resulting in phospholipidosis (PLD); however, the molecular mechanism underlying CAD-induced PLD remains to be resolved. Here, we found that treatment of normal rat kidney cells with CADs known to induce PLD caused redistribution of a mannose 6-phosphate/IGF-II receptor (MPR300) from the TGN to endosomes and concomitantly increased the secretion of lysosomal enzymes, resulting in a decline of intracellular lysosomal enzyme levels. These results enable the interpretation of why CADs cause excessive accumulation of undegraded substrates, including phospholipids in lysosomes, and led to the conclusion that the impaired MPR300-mediated sorting system of lysosomal enzymes reflects the general mechanism of CAD-induced PLD. In addition, our findings suggest that the measurement of lysosomal enzyme activity secreted into culture medium is useful as a rapid and convenient *in vitro* early screening system to predict drugs that can induce PLD.

Phospholipidosis (PLD) induced by cationic amphiphilic drugs (CADs) is characterized by the excessive intracellular accumulation of phospholipids with concentric myelin-like structures, termed lamellar bodies, which are the morphological marker of PLD [1–3]. So far, over 50 CADs, including antibiotics, antidepressants, antipsychotics, and antimalarial and antirrythmic agents have been identified to cause PLD [3]. CADs share a hydrophobic ring structure and a hydrophilic side chain with a charged cationic amine group and, owing to such a chemical structure, can directly bind to phospholipids [1]; therefore, CAD-induced PLD has been considered to be caused by the direct interaction of CADs with phospholipids.

Lysosomes are the primary intracellular compartment target, where phospholipids are stored. Because of their weak basic properties, CADs taken up by cells are protonated in the acidic environment of lysosomes, thereby becoming trapped in lysosomes [4]. Several consequences for CAD-induced PLD have been proposed: including direct inhibition of phospholipase activity by CADs, protection of phospholipids against phospholipase due to formation of the CAD–phospholipids complex, and increased synthesis of phospholipids [1,4]. Thus, although the principal mechanism responsible for the development of PLD induced by CADs has been considered to impair phospholipid metabolism, little is known about the detailed molecular mechanisms.

In the present study, we show that treatment of normal rat kidney (NRK) cells with various CADs that induce PLD causes a marked change in the localization of a mannose 6-phosphate/IGF-II receptor (MPR300), a lysosomal enzyme receptor [5], from the TGN, its functional site, to endosomes. Such an altered localization of MPR300 induces mistargeting of lysosomal enzymes, consequently leading to excessive accumulation of phospholipids.

Materials and methods

Materials. Amiodarone, amoxicillin, aspirin, captopril, chloroquine, chlorpromazine, erythromycin, gentamicin, haloperidol, imipramine, maprotiline, perhexiline, quinidine, tamoxifen, and thioridazine were purchased from Sigma–Aldrich (St. Louis, MO) and tilorone was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Antibodies. Mouse monoclonal antibodies to AP-1, EEA1, and GM130 were purchased from Transduction Laboratories (Lexington, KY). Rabbit polyclonal antibodies to rat MPR300, LGP85, LGP107, and cathepsin D were described previously [6–9]. Alexa488- and Alexa594-labeled secondary antibodies and Texas-Red dextran were purchased from Molecular Probes (Eugene, OR).

Cell culture. NRK cells were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, and 100 units of penicillin/streptomycin/ml in humidified 95% air and 5% CO_2 at 37 °C. The cells were plated onto 13 mm coverslips or 35 mm plastic dishes and, after 24 h, the cells were used for immunocytochemical and biochemical experiments.

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0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.09.121

Immunofluorescence microscopy. Cells cultured on coverslips were fixed in 4% paraformaldehyde and processed for immunofluorescence microscopy, as described previously [10,11]. Confocal images were acquired using a Zeiss confocal microscope (LSM 510 META). All images were created using Adobe Photoshop CS (Adobe Systems, San Jose, CA).

Western blotting. Cells were extracted using RIPA buffer (20 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 0.5% Triton X-100, 0.1% SDS, 1 mM EDTA, 10 mM NaF, and 1 mM Na_3VO_4). The lysates were subjected to SDS–PAGE and proteins were transferred to a PVDF membrane (Millipore Corp.) using a semi-dry blotting system. The membranes were blocked with 5% skimmed milk powder and incubated with anti-rat MPR300 antibody followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (Amersham Biosciences). Cathepsin D was immnunoprecipitated from cell lysates and media, as described previously [8,12]. Immunoprecipitated cathepsin D was detected using ReliaBLOT HRP Conjugate (Bethyl Laboratories). Blots were developed by ECL (Amersham Biosciences) and signals were visualized using Fuji LAS1000.

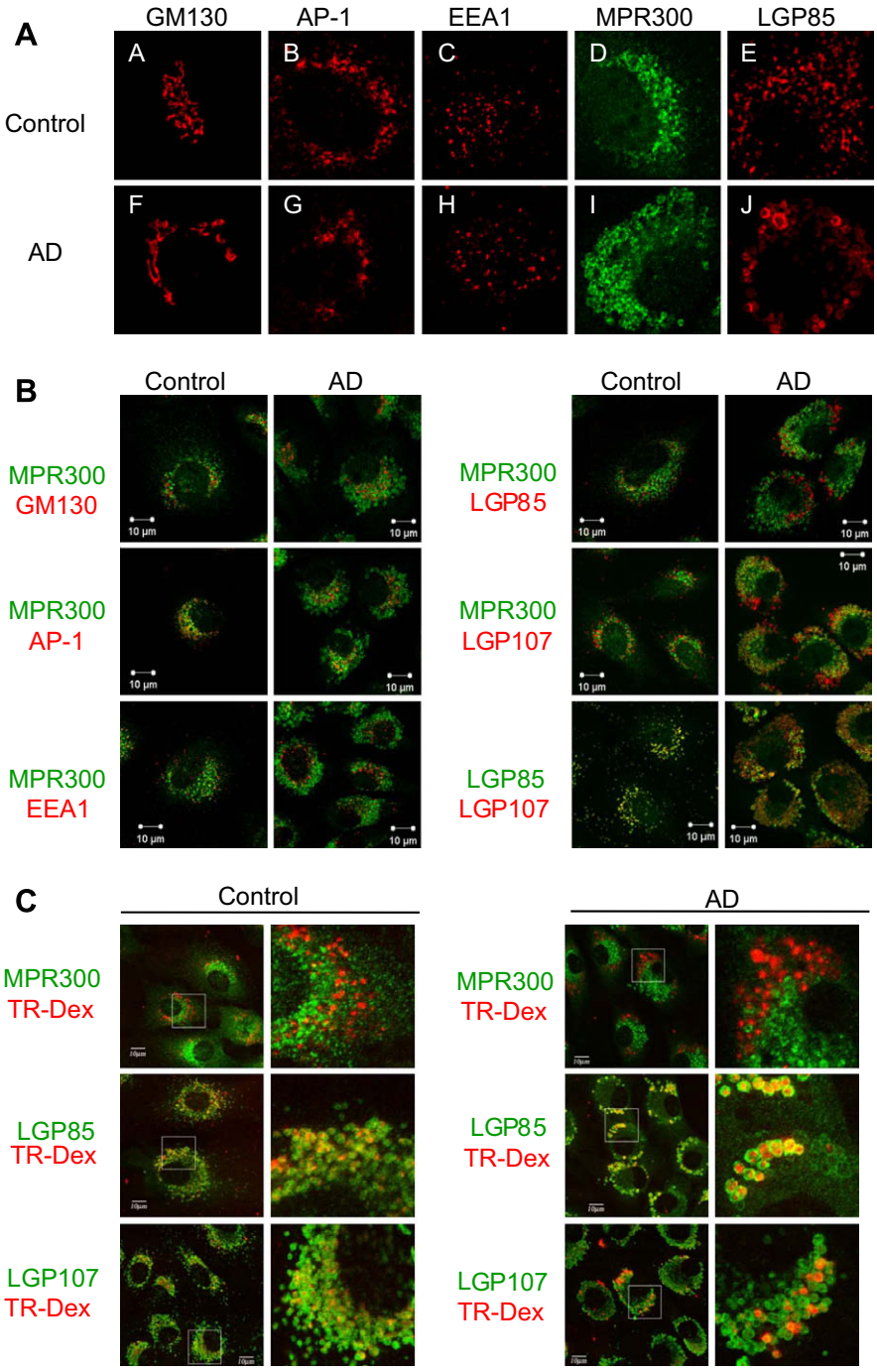

Fig. 1. Amiodarone-induced redistribution of MPR300 from the TGN to endosomes. (A) NRK cells were incubated with 1% DMSO (control) or 20 μM amiodarone (AD) for 24 h. After incubation, cells were fixed, permeabilized, and immunostained for GM130, EEA1, AP-1, MPR300, and LGP85. Cells were visualized by confocal microscopy. (B) Cells treated with or without 20 μM AD for 24 h were double-labeled for MPR300 and GM130, AP-1, EEA1, LGP85, or LGP107 or LGP85 and LGP107, and visualized by confocal microscopy (C) To label lysosomes, cells were incubated with 0.1 mg/ml of Texas-Red dextran (TR-Dex) for 6 h and chased for 6 h. Cells were then incubated with or without 20 μM AD for 24 h, and processed for immunostaining using antibodies against MPR300, LGP85, and LGP107. Cells were visualized by confocal microscopy.

Lysosomal enzyme assays. β-Hexosaminidase activities were determined as described by Umeda et al. [13], using 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside (Sigma–Aldrich) as the substrate. Cathepsin D activities were assayed as described by Yasuda et al. [14], using MOCAc-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH_2 (Peptide Institute Inc., Osaka, Japan) as the substrate.

Results

Amiodarone treatment causes the altered distribution of MPR300

To explore the mechanism of CAD-induced PLD, we first used amiodarone, which has been extensively examined in studies of CAD-induced PLD. When NRK cells treated with 20 μM amiodarone for 24 h were processed for immunostaining with several organelle-specific antibodies, we found that amiodarone treatment caused a marked change in the distribution of MPR300. We have previously demonstrated that MPR300 is primarily localized to the TGN in NRK cells [10], as well as in rat 3Y-1 [6], human skin fibroblasts [13], and COS cells [7]. As shown in Fig. 1A, the TGN-like localization of MPR300, which is seen as reticular and small punctuate structures around the perinuclear region in control NRK cells, was altered to numerous large spherical vesicles after treatment with amiodarone. By contrast, amiodarone did not influence the distribution of other organelle-specific marker proteins, such as early endosome marker EEA1, *cis*-Golgi marker GM130, and TGN marker AP-1. Since amiodarone treatment did not affect the distribution of AP-1, it is suggested that the altered distribution of MPR300 by amiodarone is not merely due to destruction of the TGN structure. Amiodarone treatment also caused the enlargement of vesicles positive for LGP85, a late endosomal and lysosomal membrane protein [7,15], presumably reflecting the excessive accumulation of phospholipids.

Amiodarone treatment causes redistribution of MPR300 from the TGN to late endosomes

We next examined the characteristics of amiodarone-induced MPR300-positive large vesicles. As shown in Fig. 1B, enlarged MPR300-positive vacuoles in cells treated with amio-

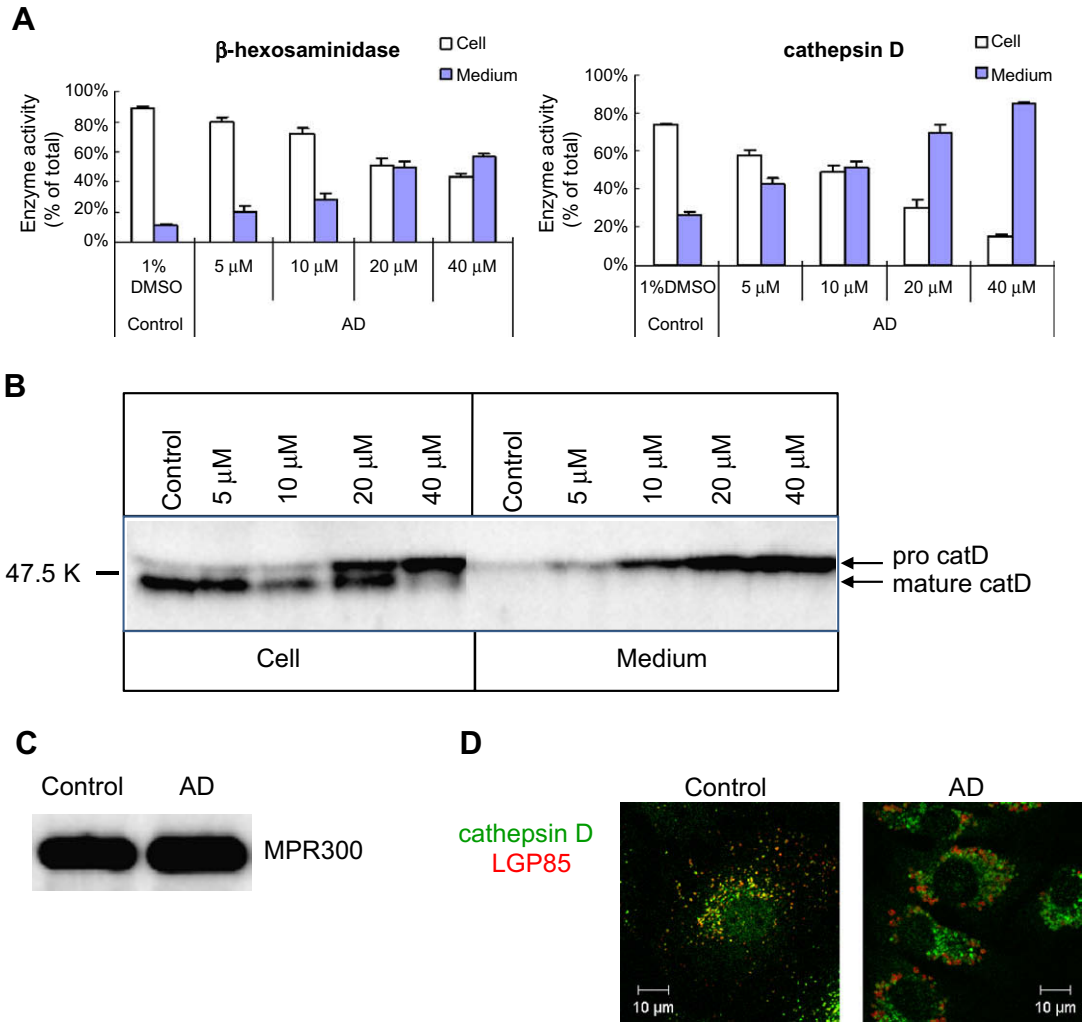

Fig. 2. Amiodarone-induced hypersecretion of lysosomal enzymes. Cells were incubated for 24 h in the presence or absence of AD with the indicated concentration. After incubation, activities of β-hexosaminidase and cathepsin D were determined in cells and media. Secretion activity as a percentage of total activity is given. Error bars represent the means ±SE ($n = 3$). (B) Cells were incubated for 24 h in the presence or absence of AD with the indicated concentration. After incubation, cathepsin D was immunoprecipitated from cells and media. Immunoprecipitates were subjected to SDS-PAGE, followed by Western blot analysis. (C) An equal amount of cell lysates from control and AD-treated cells was subjected to SDS-PADE, followed by Western blot analysis for MPR300. (D) Cells were incubated with 1% DMSO (control) or 20 μM amiodarone for 24 h. After incubation, cells were fixed, permeabilized, double-labeled for cathepsin D and LGP85, and visualized by confocal microscopy.

darone never colocalized with EEA1, AP-1, and GM130. Partial colocalization was observed with LGP85; however, MPR300 staining was negative for most of the enlarged vacuoles with strong fluorescence intensity of LGP85. Instead, it was visible in large vacuoles exhibiting relatively weak staining for LGP85. On the other hand, in cells treated with amiodarone, MPR300 staining extensively overlapped LGP107 compared with LGP85. This is somewhat surprising because LGP107 is also known as a late endosomal and lysosomal membrane protein [9], similar to LGP85. Indeed, LGP85 and LGP107 almost completely overlapped in control cells; however, in amiodarone-treated cells, LGP107-positive large vacuoles located in a relatively proximal region to the nucleus displayed weak staining for LGP85, which resembled the staining pattern between LGP85 and MPR300. On the other hand, when cells previously preloaded with Texas-red dextran to label lysosomes were further incubated for 24 h with amiodarone, all Texas-red dextran-loaded lysosomes exclusively colocalized with large vacuoles possessing strong intensity for LGP85, but were negative for MPR300 (Fig. 1C). Similarly, in amiodarone-treated cells, Texas-red dextran-positive vacuoles also completely overlapped LGP107-positive vacuoles, whereas the majority of LGP107-positive large vacuoles lacked Texas-red dextran. These results suggest that amiodarone-induced MPR300-positive large vacuoles are derived from late endosomes. Furthermore, the different distribution between LGP85 and LGP107 observed in amiodarone-treated cells may be correlated with or account for our previous findings that LGP85 and LGP107 are targeted to late endosomes/lysosomes via a different pathway [9].

Amiodarone treatment causes the enhanced secretion of lysosomal enzymes

Newly synthesized lysosomal enzymes acquire mannose 6-phosphate residues in the Golgi complex and are recognized in the TGN by two mannose 6-phosphate receptors, MPR300 and MPR46 [5]. The ligand-receptor complex is subsequently packed into clathrin-coated vesicles at the TGN and targeted to endosomal compartments. The acidic environment of endosomes causes the release of enzymes from MPRs, after which lysosomal enzymes are transferred to lysosomes while MPRs are retrieved to the TGN for further rounds of sorting. Thus, MPRs are responsible for selective sorting of lysosomal enzymes from secretory proteins. We then assessed whether the altered distribution of MPR300 induced by amiodarone treatment results in impaired targeting of lysosomal enzymes to lysosomes. To this end, NRK cells were treated for 24 h with the indicated concentrations of amiodarone and β-hexosaminidase, and cathepsin D activities were determined in both cells and media. As shown in Fig. 2A, in amiodarone-treated cells, secretion of β-hexosaminidase and cathepsin D was significantly elevated in a dose-dependent manner. Similar enhanced secretion of β-mannosidase, β-galactosidase, and β-glucuronidase was also observed in cells treated with amiodarone (data not shown). Western blot analyses further revealed that amiodarone treatment inhibits processing of the precursor form of cathepsin D to the mature form and increases secretion of the precursor form in a dose-dependent manner (Fig. 2B). Our results further suggest that the enhanced secretion of lysosomal enzymes is not due to increased degradation of MPR300 by redistribution to late endosomes, because the amount of MPR300 was not reduced by amiodarone treatment (Fig. 2C). Therefore, these results suggest that amiodarone impairs the biosynthetic trafficking pathway of lysosomal enzymes, due to blockade of the retrieval pathway of MPR300 from late endosomes to the TGN, leading to enhanced secretion of the precursor form of lysosomal enzymes.

Table 1
Effect of various compounds on MPR300-dependent lysosomal targeting of β-hexosaminidase.

Compound	Dose (μM)	(Ratio of control)	Enlargement	
			MPR300-positive compartment	LGP85-positive compartment
Amiodarone	5	1.7	±	±
	10	2.4	+	+
	20	5.2	+	+
	40	5.5	+	+
Amoxicillin	1000	1.1	−	−
	2000	1.1	−	−
Aspirin	1000	1.1	−	−
	2000	1.0	−	−
Captopril	1000	1.1	−	−
	2000	1.1	−	−
Chloroquine	5	4.2	+	+
	10	5.3	+	+
	20	6.0	+	+
	40	7.8	+	+
Chlorpromazine	5	1.2	NE	NE
	10	1.4	±	+
	20	1.7	+	+
Erythromycin	200	1.4	±	±
	400	2.6	+	+
	800	4.6	+	+
Gentamicin	1000	1.0	−	−
	2000	1.2	−	+
	4000	1.0	−	+
Haloperidol	10	3.1	+	+
	20	5.3	+	+
	40	6.2	+	+
Imipramine	10	1.1	±	±
	20	1.2	+	+
	40	1.9	+	+
	80	4.3	+	+
Maprotiline	2.5	1.1	±	+
	5	1.4	±	+
	10	1.6	±	+
	20	2.5	+	+
Perhexiline	1.25	1.2	±	+
	2.5	1.3	+	+
	5	1.8	+	+
Quinidine	10	NE	−	−
	100	3.2	+	+
Tamoxifen	2.5	1.4	±	+
	5	1.4	±	+
	10	2.3	+	+
	20	6.4	+	+
Thioridazine	2.5	1.0	±	+
	5	1.3	±	+
	10	2.5	+	+
Tilorone	2.5	2.1	+	+
	5	2.9	+	+
	10	4.9	+	+
	20	6.2	+	+

−, no change; ±, slight; +, apparent; NE, not examined.

PLD-inducible CADs impair the MPR300-mediated lysosomal targeting of β-hexosaminidase

To further examine whether other CADs that are known to induce PLD also cause the redistribution of MPR300 and hypersecterion of lysosomal enzymes, we used 15 additional compounds known to induce PLD or not. Table 1 summarizes the ratio of secretion of β-hexosaminidase by these compound treatments. When cells were treated with drugs not inducing PLD, i.e., amoxicillin, aspirin, and captopril, neither the enhanced secretion of lysosomal enzymes nor the alteration of MPR300 distribution was induced (Table 1 and Fig. 3A). Increased secretion of β-hexosaminidase activities was observed in cells treated with 11 of the 12 positive CADs tested (except gentamicin) in a concentration-dependent

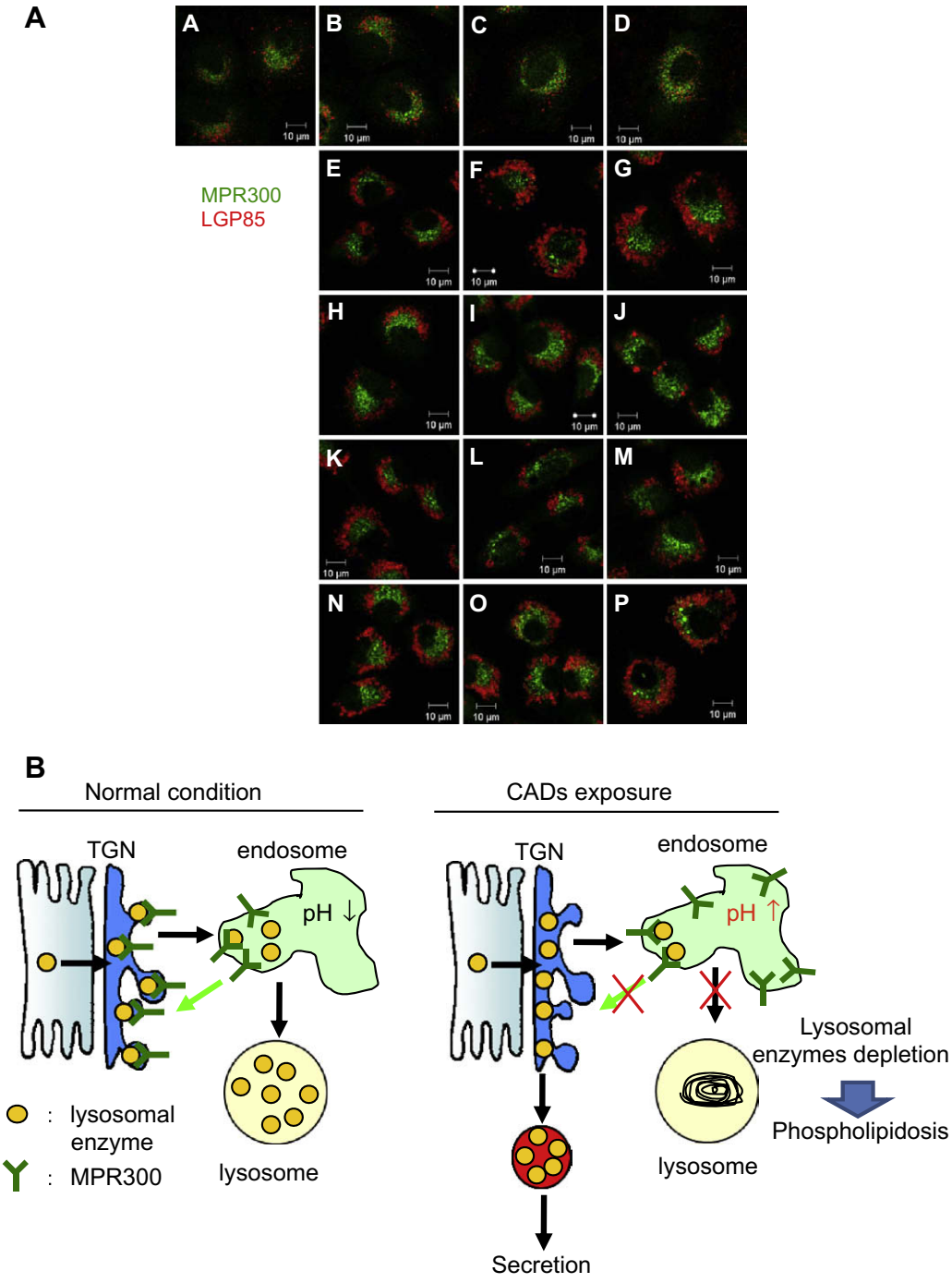

Fig. 3. PLD-inducing drugs altered MPR300 distribution. (A) Cells were incubated for 24 h with various compounds; 1% DMSO (A), 2 mM amoxicillin (B), 2 mM aspirin (C), 2 mM captopril (D), 20 μM chlorpromazine (E), 20 μM chloroquine (F), 400 μM erythromycin (G), 4 mM gentamicin (H), 20 μM haloperidol (I), 40 μM imipramine (J), 10 μM maprotiline (K), 5 μM perhexiline (L), 100 μM quinidine (M), 10 μM tamoxifen (N), 10 μM thioridazine (O), and 10 μM tilorone (P) and then cells were fixed, permeabilized, and double-labeled for MPR300 and LGP85. Cells were visualized by confocal microscopy. (B) Schematic hypothesis of the mechanism of CAD-induced PLD.

manner. In addition, the concentration required for the increased secretion of β-hexosaminidase differed depending upon the compounds used; quinidine and erythromycin were required at 10- to 100-fold higher concentrations than other compounds, consistent with a previous report [16].

The altered distribution of MPR300 and enlargement of LGP85-positive large vesicles were also observed in cells treated with CADs known to induce PLD, excluding gentamicin, although gentamicin slightly enlarged LGP85-positive vacuoles. Because gentamicin has been reported to induce PLD in the kidney [17], a mechanism of PLD induced by gentamicin might differ from that by other CADs. It should be noted here that the distribution pattern of MPR300 caused by chloroquine or tilorone differed from that by other CADs: MPR300-positive large vacuoles formed by chloroquine or tilorone increased in size, but decreased in number as compared with those by other CADs. Chloroquine- or tilorone-induced MPR300-positive large vacuoles colocalized with the early endosome marker EEA1, in accordance with our previous results that chloroquine resulted in the redistribution of MPR300 to EEA1-positive early endosomes in human skin fibroblasts [12]. Taken together, our findings appar-

ently indicate that CADs known to induce PLD disrupt the MPR300-dependent lysosomal targeting pathway of lysosomal enzymes.

Discussion

In the present study, we demonstrated that CADs cause the redistribution of MPR300 from the TGN to endosomes and the concurrent enhanced secretion of lysosomal enzymes, and as a result of the reduction in the level of intracellular lysosomal enzymes, result in the accumulation of phospholipids as well as various substrates which are degraded in lysosomes (Fig. 3B). Since these phenomena were observed in cells treated with all CADs known to induce PLD, dysfunction of the MPR300-mediated lysosomal enzyme targeting system could account for the universal mechanism of CAD-induced PLD. The enhanced secretion of multiple lysosomal enzymes caused by CAD treatment therefore resembles the phenotype observed in fibroblasts of mucolipidosis II and III patients [18], which are rare autosomal recessive inherited lysosomal storage disorders due to deficient UDP-*N*-acetylglucosamine: lysosomal hydrolase *N*-acetylglucosamine-1-phosphotransferase [19]. Because lysosomal acid phospholipase is also targeted to lysosomes via a mannose 6-phosphate-dependent pathway [18], our findings lead to the speculation that the effect of CADs on phospholipid accumulation might be predominantly ascribed to the reduction of intracellular lysosomal enzymes, including phospholipase, due to the impairment of MPR300 trafficking, rather than the interference of phospholipid metabolism, including direct inhibition of phospholipase activity. In addition, our data are consistent with, and provide an explanation for, a previous report on the increased release of β-hexosaminidase in amiodarone-treated rabbit alveolar macrophages [20].

What is the molecular mechanisms underlying CAD-induced redistribution of MPR300 from the TGN to endosomes? It has been considered that trafficking of MPR300 between the TGN and endosomes depends on AP-1 complex, because targeted disruption of the μ1A-subunit caused the redistribution of MPR300 from the TGN to early endosomes and enhanced the secretion of lysosomal enzymes [21]. One possibility is therefore that CADs might block the AP-1-mediated retrieval pathway of MPR300 from endosomes to the TGN. In this context, our results showed that amiodarone treatment did not significantly affect the distribution (Fig. 1B) and the expression level of AP-1 (data not shown), thereby suggesting that the altered distribution of MPR300 observed in CAD-treated cells is not due to impaired AP-1 function. However, we cannot completely rule out the possibility of AP-1 dysfunction by CADs, because recent gene expression analysis using DNA microarrays on HepG2 cells treated with compounds known to induce PLD has demonstrated down-regulation of the σ1-subunit gene of AP-1 adaptor complex [22].

Another possibility is that increased endosomal pH by CADs might block the recycling of MPR300 to the TGN by impairing the dissociation of receptor-ligand complexes, leading to missorting of lysosomal enzymes to extracellular space, since CADs can raise the pH of acidic compartments [1]. This notion may be also supported by the results that in addition to the inhibition of proteolytic processing of procathepsin D, which is an indicator of disrupted acidification, cathepsin D retained intracellulary was localized in the compartment where MPR300 was redistributed in cells treated with CADs, although endosomal compartments, where cathepsin D and MPR300 were colocalized, differed according to the CADs used, i.e., late endosomes in amiodarone (Fig. 2D) versus early endosomes in chloroquine and tilorone (data not shown).

Nevertheless, there was good correlation of the potential of CADs between the impairment of MPR300-dependent lysosomal enzyme targeting and the induction of PLD, thereby suggesting that the assay of secreted lysosomal enzyme activities is a useful screening method to predict drugs capable of inducing PLD. So far, different *in vitro* screening systems for drug-induced PLD have been reported; these include measurement of the accumulation of the fluorescent-labeled phospholipid probe (NBD-PC, NBD-PE, and LipidTox) and fluorescence dye (Nile Red) using fluorescence microscopy, flow cytometry, or fluorometry [23–26] and identification of potential biomarkers of PLD based on gene expression analysis [16,22,27,28]. Because drug-induced PLD is an important issue in the development of new drugs, development of a rapid and reliable screening system to facilitate or predict new drugs for PLD at an early development stage is required. From this point of view, the measurement of secreted lysosomal enzyme levels using NRK cells developed in this study can provide a more rapid and conventional screening system for drug-induced PLD than the assay systems mentioned above. Furthermore, our assay system may be useful in not only an *in vitro*, but also an *in vivo* screening system for validation of PLD potential in new drug development.

Acknowlegments

We thank Dr. Kenji Akasaki (Fukuyama University, Japan) for anti-LGP85 monoclonal antibodies. This study was supported by an Astellas Research Collaboration Fund.

References

[1] U.P. Kodavanti, H.M. Mehendale, Cationic amphiphilic drugs and phospholipid storage disorder, Pharmacol. Rev. 42 (1990) 327–354.
[2] M. Reasor, S. Kacew, Drug-induced phospholipidosis: are there functional consequence?, Exp. Biol. Med. (Maywood) 226 (2001) 825–830.
[3] N. Anderson, J. Borlak, Drug-induced phospholipidosis, FEBS Lett. 580 (2006) 5533–5540.
[4] W.H. Halliwell, Cationic amphiphilic drug-induced phospholipidosis, Toxicol. Pathol. 25 (1997) 53–60.
[5] S. Kornfeld, Structure and function of the mannose 6-phosphate/insulin-like growth factor II receptors, Annu. Rev. Biochem. 61 (1992) 307–330.
[6] K. Hirosako, H. Imasato, Y. Hirota, T. Kuronita, N. Masuyama, M. Nishioka, A. Umeda, H. Fujita, M. Himeno, Y. Tanaka, 3-Methyladenine specifically inhibits retrograde transport of cation-independent mannose 6-phosphate/insulin-like growth factor II receptor from the early endosome to the TGN, Biochem. Biophys. Res. Commun. 316 (2004) 845–852.
[7] T. Kuronita, E.L. Eskelinen, H. Fujita, P. Saftig, M. Himeno, Y. Tanaka, A role for the lysosomal membrane protein LGP85 in the biogenesis and maintenance of endosomal and lysosomal morphology, J. Cell Sci. 115 (2002) 4117–4131.
[8] Y. Tanaka, G. Guhde, A. Suter, E.L. Eskelinen, D. Hartmann, R. Lüllmann-Rauch, P.M. Janssen, J. Blanz, K. von Figura, P. Saftig, Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice, Nature 406 (2000) 902–906.
[9] K. Niwa, R. Tanaka, H. Murase, T. Ishikawa, H. Fujita, M. Himeno, Y. Tanaka, Two lysosomal membrane proteins, LGP85 and LGP107, are delivered to late endosomes/lysosomes through different intracellular routes after exiting from the trans-Golgi network, Biochem. Biophys. Res. Commun. 301 (2003) 833–840.
[10] Y. Hirota, N. Masuyama, T. Kuronita, H. Fujita, M. Himeno, Y. Tanaka, Analysis of post-lysosomal compartments, Biochem. Biophys. Res. Commun. 314 (2004) 306–312.
[11] Y. Hirota, T. Kuronita, H. Fujita, Y. Tanaka, A role of Rab5 activity for biogenesis and maintenance of endosomal/lysosomal compartments, Biochem. Biophys. Res. Commun. 364 (2007) 40–47.
[12] Y. Tanaka, R. Tanaka, T. Kawabata, Y. Noguchi, Y.M. Himeno, Lysosomal cysteine protease, cathepsin B, is targeted to lysosomes by the mannose 6-phosphate-independent pathway in rat hepatocytes: site-specific phosphorylation in oligosaccharides of the proregion, J. Biochem. 128 (2000) 39–48.
[13] A. Umeda, H. Fujita, T. Kuronita, K. Hirosako, M. Himeno, M.Y. Tanaka, Distribution and trafficking of MPR300 is normal in cells with cholesterol accumulated in late endocytic compartments: evidence for early endosome-to-TGN trafficking of MPR300, J. Lipid Res. 44 (2003) 1821–1832.
[14] Y. Yasuda, T. Kageyama, A. Akamine, M. Shibata, E. Kominami, Y. Uchiyama, K. Yamamoto, Characterization of new fluorogenic substrates for the rapid and sensitive assay of cathepsin E and cathepsin D, J. Biochem. (Tokyo) 125 (1999) 1137–1143.
[15] T. Kuronita, T. Hatano, A. Furuyama, Y. Hirota, N. Masuyama, P. Saftig, M. Himeno, H. Fujita, Y. Tanaka, The NH_2 -terminal transmembrane and lumenal domains of LGP85 are needed for the formation of enlarged endosomes/lysosomes, Traffic 6 (2005) 895–906.
[16] F. Atienzar, H. Gerets, S. Dufrane, K. Tilmant, M. Cornet, S. Dhalluin, B. Ruty, G. Rose, M. Canning, Determination of phospholipidosis potential based on gene expression analysis in HepG2 cells, Toxicol. Sci. 96 (2007) 101–114.

[17] M.P. Mingeot-Leclercq, G. Laurent, P.M. Tulkens, Biochemical mechanism of aminoglycoside-induced inhibition of phosphatidylcholine hydrolysis by lysosomal phospholipases, Biochem. Pharmacol. 37 (1988) 591–599.
[18] S.M. Jansen, J.E.M. Groener, B.J.H.M. Poorthuis, Lysosomal phospholipase activity is decreased in mucopilidosis II and III fibroblasts, Biochim. Biophys. Acta 1436 (1999) 363–369.
[19] M.L. Reitman, A. Varki, S. Kornfeld, Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in uridine 5′-diphosphate *N*-acetylglucosamine: glycoprotein *N*-acetylglucosaminylphosphotransferase activity, J. Clin. Invest. 67 (1981) 1574–1579.
[20] A. Baritussio, S. Marzini, M. Agostini, A. Alberti, C. Cimenti, D. Bruttomesso, E. Manzato, D. Quaglino, A. Pettenazzo, Amiodarone inhibits lung degradation of SP-A and perturbs the distribution of lysosomal enzymes, Am. J. Physiol. Lung Cell Mol. Physiol. 281 (2001) L1189–L1199.
[21] C. Meyer, D. Zizioli, S. Lausmann, E.L. Eskelinen, J. Hamann, P. Saftig, K. von Figura, P. Schu, mu1A–Adaptin-deficient mice: lethality, loss of AP-1 binding and rerouting of mannose 6-phosphate receptors, EMBO J. 19 (2000) 2193–2203.
[22] H. Sawada, K. Takami, S. Asahi, A toxicogenomic approach to drug-induced phospholipidosis: analysis of its induction mechanism and establishment of a novel *in vitro* screening system, Toxicol. Sci. 83 (2005) 282–292.
[23] A. Casartelli, M. Bonato, P. Cristofori, F. Crivellente, G. Dal Negro, I. Masotto, C. Mutinelli, K. Valko, V. Bonfante, A cell-based approach for the early assessment of the phospholipidogenic potential in pharmaceutical research and drug development, Cell Biol. Toxicol. 19 (2003) 161–176.
[24] T. Kasahara, K. Tomita, H. Murano, T. Harada, K. Tsubakimoto, T. Ogihara, S. Ohnishi, C. Kakinuma, Establishment of an in vitro high-throughput screening assay for detecting phospholipidosis-inducing potential, Toxicol. Sci. 90 (2006) 133–141.
[25] H. Fujimura, E. Dekura, M. Kurabe, N. Shimazu, M. Koitabashi, W. Toriumi, Cell-based fluorescence assay for evaluation of new-drugs potential for phospholipidosis in an early stage of drug development, Exp. Toxicol. Pathol. 58 (2007) 375–382.
[26] N. Bhandari, D.J. Figueroa, J.W. Lawrence, D.L. Gerhold, Phospholipidosis assay in HepG2 cells and rat or rhesus hepatocytes using phospholipid probe NBD-PE, Assay Drug Dev. Technol. 6 (2008) 407–419.
[27] P. Nioi, B.K. Perry, E.J. Wang, Y.Z. Gu, R.D. Snyder, In vitro detection of drug-induced phospholipidosis using gene expression and fluorescent phospholipid-based methodologies, Toxicol. Sci. 99 (2007) 162–173.
[28] M. Hirode, A. Ono, T. Miyagishima, T. Nagao, Y. Ohno, T. Urushidani, Gene expression profiling in rat liver treated with compounds inducing phospholipidosis, Toxicol. Appl. Pharmacol. 229 (2008) 290–299.