Functions for Ether Phospholipids in Animal Cells

Abstract

Ether phospholipids make up a large portion of the phospholipid mass in humans yet little is known concerning their role(s) in cellular processes. One approach to determining these function(s) is to isolate and study somatic cell mutants which are deficient in ether phospholipids. A description of the mutant cell lines which have been isolated, and the information that we have gained using them is described.

Introduction

Animal cell membranes display phospholipid heterogeneity not only with respect to the headgroup and fatty acid constituents, but also the manner in which the aliphatic chain is attached at the *sn*-1 position of the glycerol backbone. Figure 1 illustrates the structures of the three forms in which phospholipids can exist in animal cells using ethanolamine phospholipids as an example. These are 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine (phosphatidylethanolamine), 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (plasmanylethanolamine), and 1-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (plasmenylethanolamine). Phosphatidylethanolamine contains two long-chain fatty acids attached through ester bonds at the *sn*-1 and *sn*-2 carbons of the glycerol backbone. The latter two phospholipid species differ from phosphatidylethanolamine in that they contain an ether-linked long-chain fatty alcohol at the *sn*-1 position. Plasmenylethanolamine can be differentiated from plasmanylethanolamine due to the addition of a *cis* double bond between the first and second carbons of the fatty alcohol moiety. Ethanolamine and choline phospholipids are the only phospholipids in which appreciable amounts exist in the ether-linked form. Less than 1% of the serine and inositol phospholipids are ether-linked [15]. In most tissues, the dominant ether-linked ethanolamine phospholipid is plasmenylethanolamine with little or no plasmanylethanolamine present [15]. In the case of choline phospholipids the opposite is true, with the plasmanyl varient dominating. An exception to this latter case is human cardiac tissue where plasmenylcholine is a major component (40%) of the choline phospholipids [15]. Overall, plasmalogens (the common name for the plasmenyl variant] are the dominant ether

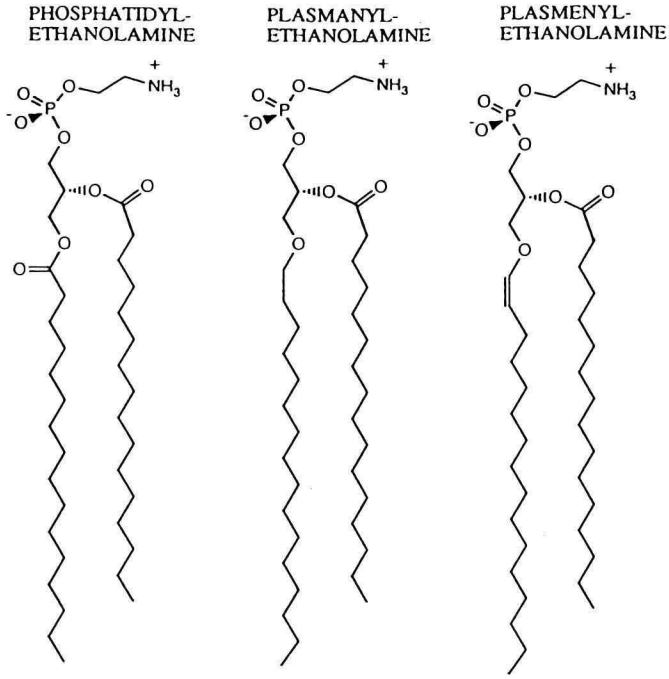

Fig. 1. Ethanolamine head-group species found in animal cell membranes. The analogous choline phospholipids are phosphatidyl-, plasmanyl- and plasmenylcholine.

phospholipid, constituting approximately 19% of the total phospholipid mass in humans [15]. Depending upon the tissue and the animal species, up to 75% of the ethanolamine- and/or choline-linked phospholipid of an animal cell may be recovered as the plasmenyl variant.

Despite the abundance of ether lipids, little is known about their physiological and cellular roles. Although the role of plasmanylcholine as the direct precursor during the biosynthesis of platelet activating factor (PAF; 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), a potent hormone [30], is well established, functions for plasmalogens have not been confirmed. Support for the importance of ether lipids in cellular function comes from descriptions of human genetic disorders in which the patients' tissues are lacking ether lipids [16,17]. The neuromuscular abnormalities associated with these patients suggest a role for ether lipids in the function of nervous and muscle tissues (which normally contain very high levels of ether lipids). The primary lesion in most of these patients is the loss of peroxisomes, an intracellular organelle which performs a variety of functions, and the role of ether lipid-deficiency in the pathophysiology of these disorders is not clear. There is a report, however, of a patient displaying similar neuromuscular dysfunctions whose fibroblasts are defective in ether lipid biosynthesis yet have normal, apparently functional peroxisomes [31].

To suggest possible roles for ether lipids one must first consider their structure and chemistry. The ether bond and the vinyl-ether bond (found in plasmalogens) give the ether-linked phospholipids unique physical and chemical characteristics. The loss of the carbonyl moiety results in altered packing within the phospholipid bilayer [18,25] and these phospholipids form non-bilayer structures at lower temperatures than their diacyl counterparts, assuming similar head group. This aspect of ether phospholipids is covered more completely by Van den Bosch elsewhere in this volume.

The chemical characteristics of the 1-alkyl and the 1-alk-1'-enyl groups are quite different, suggesting different functions for the lipid species displaying these groups. The ether bond found in the plasmanyl varient is resistant to both acid and base hydrolysis and it also renders the *sn*-1 position resistant to enzymatic cleavage by cellular phospholipase A1 activities. Based on these characteristics one might expect ether lipids to function as a tool by which the cell preserves membrane integrity during chemical insult or conditions in which certain phospholipases are activated. The vinyl-ether bond dramatically alters the chemical characteristics of plasmenyl phospholipids. Placement of a *cis* double bond adjacent to the electron-withdrawing oxygen renders the vinyl-ether group extremely sensitive to attack by a variety of insults including acid [22], mercuric ion [14] and active oxygen species (e.g. singlet oxygen; ref. 2), making this ether phospholipid species a possible target within the cell during chemical stress.

The presence of high levels of ether phospholipids in brain [14, 22], myocardium [8, 12] macrophages [24], and neutrophils [21, 27] suggests that this lipid type is important in the proper functioning of these tissues. Proposed functions for ether lipids include involvement in arachidonic acid release and prostaglandin biosynthesis [6], endogenous protection of the cell against damage by active oxygen species [19, 34], and proper expression and functioning of phosphatidylinositol-glycan-linked cell-surface proteins [26]. The known physical and chemical characteristics of ether phospholipids support some of the proposed functions mentioned above, but definitive proof has been difficult to attain.

Somatic Cell Mutants Deficient in Ether Phospholipids

In an effort to identify cellular function(s) for ether phospholipids we have chosen to generate and study somatic cell mutants which are deficient in this lipid type. The general plan is to examine the performance of a variety of cellular functions in the ether lipid-deficient mutants and the parent strain and to identify functions that are altered in the mutants, thus establishing a link between the lipid and cell function(s).

The first series of ether lipid-deficient mutants were isolated using the Chinese hamster ovary-derived cell line, CHO-K1. This is an immortal, fibroblast-like cell line which has been used previously to generate a number of mutants displaying varient phenotypes. The CHO cell line possesses a number of traits that

make it useful for mutant isolation. It is immortal, grows rapidly, can grow clonally (genetically pure isolates are obtainable) and it is genetically stable. In CHO-K1 cells, approximately 35% of the ethanolamine phospholipid fraction is plasmenylethanolamine with no plasmanyl form detectable; approximately 10% of the phospholipid mass is plasmalogen in wild-type CHO cells.

To isolate ether-lipid deficient cells, mutagenized populations of cells were screened for the absence of peroxisomal dihydroxyacetonephosphate acyltransferase (pDHAPAT), the first step in the biosynthesis of plasmenylethanolamine (Fig. 2), using colony autoradiography [32]. Three such mutant lines were obtained from separate mutagenized populations. These mutants were approximately 90% reduced in pDHAPAT activity, plasmenylethanolamine biosynthesis, and plasmalogen content. Upon further analysis it was found that alkyl-DHAP synthase activity, which catalyzes the second step in the biosynthesis of plasmenylethanolamine, was also reduced. The primary lesion in all three of these mutants was not ether lipid-deficiency, but rather an inability to properly assemble peroxisomes. Since the first 2 steps in the biosynthesis of plasmenylethanolamine are catalyzed by peroxisomal enzymes (pDHAPAT and alkyl-DHAP synthase), ether lipid biosynthesis was severely reduced. Complementation analyses showed that all three mutants were defective in the same gene (same complementation group; reference 35) and this gene, PAF-1, has been isolated and sequenced using gene-mediated complementation [28].

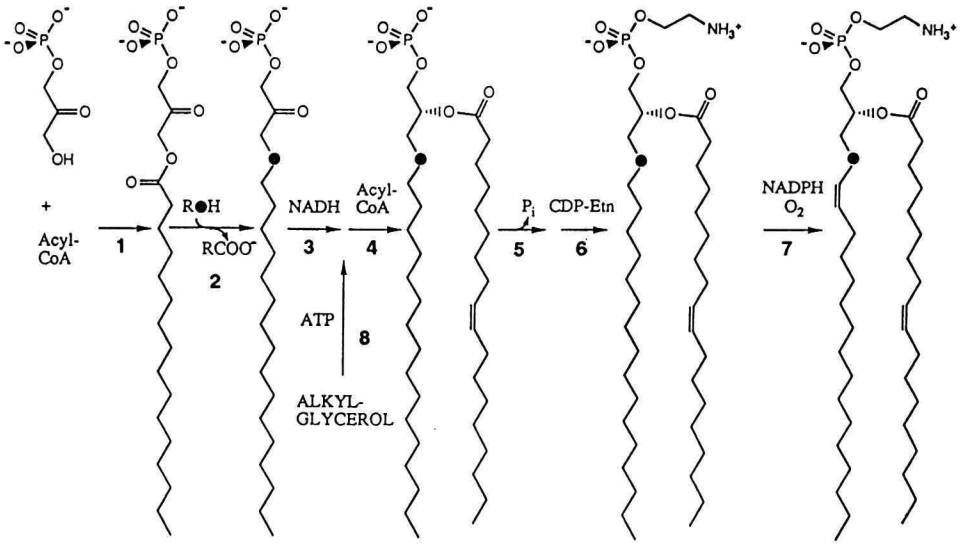

Fig. 2. Biosynthesis of plasmenylethanolamine in animal cells. The enzymatic activities which catalyze each step are: 1, peroxisomal dihydroxyactonephosphate acyltransferase (pDHAPAT); 2, alkyl-dihydroxyacetonephosphate synthase; 3, alkyldihydroxyactone-phosphate reductase; 4, 2-lyso-phosphatidate acyltransferase; 5, phosphatidate phosphohydrolase; 6, CDP-ethanolamine: diglyceride ethanolamine phosphotransferase; 7, plasmanylethanolamine desaturase (Δ1'-desaturase); 8, 1-alkylglycerol kinase. Steps 1 and 2 are peroxisomal activities while the remaining activities can be found in microsomes.

Although having ether lipid-deficient mutants in the CHO cell line was welcome and potentially useful, the lack of peroxisomes and peroxisomal functions makes interpretation of data concerning differences found in these mutants complicated. The addition of 1-alkyl-*sn*-glycerol (1-AG) to the growth medium restores ether lipids (plasmenyl-ethanolamine) to wild-type levels by entering the biosynthetic pathway downstream of the lesion (Fig. 2). Therefore, if altered functions are identified in the mutants we should be able to make a link between that function and ether lipids by examining the mutant in the presence and absence of 1-AG supplementation. Still, one must always consider peroxisome-deficiency as a possible contributor.

More recently, we isolated a series of ether lipid-deficient mutants using a murine, macrophage-like cell line, RAW 264.7, as the parent strain [36]. The RAW cells have many of the desired qualities found in the CHO cells (described above) and they perform many of the functions attributed to macrophages including PAF formation [1], prostaglandin formation [33], phagocytosis [1,37] and stimulated protein secretion [3]. The lipid composition of RAW 264.7 cells is similar to the lipid composition of CHO cells; approximately 36% of the ethanolamine phospholipids are plasmenylethanolamine while 10% of the choline phospholipids are plasmanylcholine [36].

Two of the RAW mutants, RAW.12 and RAW.108, were very deficient in ether lipid content [36], displaying severely reduced levels of both plasmenyl-ethanolamine and plasmanylcholine, but, unlike the CHO mutants, the RAW mutants contained intact, functional peroxisomes. We were able to detect intact peroxisomes through immunofluorescence microscopy and catalase activity was released from the cells only after using relatively high levels of digitonin [36]. Peroxisomal functions, such as the oxidation of very long-chain fatty acids and phytanate oxidation appeared to be normal. The lesions in the RAW mutants appeared to specifically affect steps in the biosynthetic pathway for plasmenylethanolamine. RAW.108 displayed a deficiency in peroxisomal dihydroxy-acetonephosphate acyltansferase (pDHAPAT) activity while alkyl-DHAP synthase activity, as well as all other activities in the pathway, were normal [36]. RAW.12 displayed a deficiency in both pDHAPAT and plasmanyl-ethanolamine desaturase (Δ1'-desaturase). This latter activity catalyzes the final step in plasmenylethanolamine biosynthesis, the introduction of the vinyl-ether double bond (step 7; Fig. 2). We do not know whether the dual deficiencies found in RAW.12 are a result of two separate lesions or a single lesion in a regulatory gene.

The availablity of the RAW mutants now allows us to examine questions concerning ether lipid function without the complications introduced by peroxisome deficiency. Also, the availability of RAW.12, deficient in Δ1'-desaturase, allows us to dissect out requirements for either the ether or vinyl-ether functionality. Supplementation of the growth medium with 1- alkylglycerol (1-AG) bypasses the pDHAPAT deficiency (Fig. 2) and restores plasmenylethanolamine (bearing the vinyl-ether linkage) to RAW.108 cells, but only the saturated ether lipid, plasmanylethanolamine, is restored in RAW.12 cells due to the loss of Δ1'-

Table 1. Ether Lipid-Deficient Mutants Isolated from CHO-K1 and RAW.264.7 Cell Lines

Mutant Cell Line	Parent Cell Line	Peroxisomes	Plasmalogens −1-AG	Plasmalogens +1-AG
ZR-78[1]	CHO-K1	no	no	yes
RAW.12	RAW 264.7	yes	no	no[2]
RAW.108	RAW 264.7	yes	no	yes

[1]ZR-78 is one of 3 mutants (ZR-78, ZR-82 & ZR-87) isolated using colony autoradiography [32]. All are unable to assemble peroxisomes due to a defect in the PAF-1 gene [28].
[2]RAW.12 cells accumulate plasmanylethanolamine in medium supplemented with 1-alkylglycerol [36].

desaturase activity, downstream in the pathway [36]. We should be able to distinguish between the roles of both functionalities (the ether lipid linkage and the vinyl ether linkage) in cellular processes by comparing the ability of 1-AG supplementation to restore affected processes to RAW.108 and RAW.12. For example; a decrease in stimulated arachidonate release in the ether lipid-deficient RAW mutants would suggest a role for ether lipids in this process. Restoration of wild-type-like arachidonate release in both RAW.12 and RAW.108 by 1-AG supplementation would suggest that the ether bond, alone, is required and sufficient for this process to proceed normally while restoration to normal function in only RAW.108 would suggest the requirement for the vinyl-ether functionality.

The RAW mutants were isolated using a selection procedure developed by Morand et al. [20]. We have attempted to isolate similar mutants (peroxisome$^+$/ether lipid$^-$) from mutagenized stocks of CHO cells using this procedure with no success. All of the ether lipid-deficient CHO mutants obtained by this laboratory (unpublished observations) as well as others [20] using this procedure have been deficient in peroxisome assembly as well. We are unable to explain why the same selection process, using different cell lines, yields different phenotypes, but these findings demonstrate the advantage of utilizing more than one cell line when attempting to isolate a desired mutant. Using these two cell lines we have developed a small bank of ether lipid-deficient mutants which represent defects in at least 3 genetic loci and display distinct biochemical phenotypes (Table 1). It is likely that further use of the selection protocol used to isolate the RAW mutants [36] and the use of other cell lines will yield additional mutant types.

Plasmenylethanolamine as an endogenous antioxidant?

Sensitivity to photosensitized killing:
One of the most pronounced phenotypes that has been found in the ether-lipid

deficient CHO cells has been their hypersensitivity to photosensitizer-induced cytotoxicity [34,19]. When a pyrene-containing fatty acid, 12-(1'-pyrene) dodecanoic acid (P12), is added to the growth medium it is taken up by animal cells and incorporated into complex lipids. P12-containing cells can be damaged by exposure to long-wavelength UV light (>300 nm) due the generation of singlet oxygen [9] and possibly other reactive oxygen species. This effect is titratable in that the amount of damage can be adjusted by varying any one of 3 parameters: 1) light intensity; 2) light exposure time; and 3) amount of cellular P12. We were able to find conditions, by varying any one of these factors, where the wild-type CHO cells survived while the ether lipid-deficient mutants did not [34]. Restoration of plasmenylethanolamine to the mutant cells by 1-AG supplementation partially restored wild type-like sensitivity to the mutants [34]. These data suggested that plasmenyl-ethanolamine played some role in the protection of the CHO cells against reactive oxygen species such as singlet oxygen. The report by Hoefler et al. [13] that plasmalogen-deficient human fibroblasts were also hypersensitive to photosensitized killing, and the identification of breakdown products that would be predicted during oxidative attack of plasmenylethanolamine by singlet oxygen [19], add support for this hypothesis.

Although this evidence suggested the role of plasmalogens as endogenous antioxidants, the peroxisome-deficiency phenotype associated with the CHO mutants prevented a clear interpretation. The problem stems from the fact that P12 is not only incorporated into complex lipids, but is also metabolized through beta-oxidation in the peroxisome [10]. Probably as a result of the loss of peroxisomal beta-oxidation, the mutants accumulate more P12 than the wild type cells. The hypersensitivity of the CHO mutants to P12/UV treatment was likely due to a combination of the loss of both plasmenylethanolamine and peroxisomal beta-oxidation. This would explain only a partial rescue of the mutants by 1-AG supplementation. Similar observations were made by Hoefler et al. [13] using peroxisome/plasmalogen-deficient human fibroblasts.

Chemically induced hypoxia:

In an effort to circumvent complications inherent in the P12/UV model, we have chosen another method by which we could expose the cells to oxidative damage without using a metabolizable sensitizer (such as P12). In chemically induced anoxia, cells are exposed to cyanide, uncoupling the electron transport system, and eventually depleting ATP stores [11]. Several events occur as a result of this treatment. Dawson et al. have shown that, similar to episodes of oxygen depletion (hypoxia) in organs, cyanide induced hypoxia caused the generation of reactive oxygen species in culture rat hepatocytes prior to cell death [7]. The generation of a reactive oxygen species such as superoxide is presumably due to the direct transfer of electrons from the respiratory chain to molecular oxygen [29]. Superoxide can also be used to generate other reactive species. Corey et al. [5] have postulated that, under the proper conditions superoxide can be oxidized to singlet oxygen in a biological system. These reactive oxygen species contribute to cell damage and, ultimately, to cell death.

If plasmalogens do serve a role in protecting the cell against oxidative damage, one might expect that the plasmalogen-deficient RAW cells would be more sensitive to chemically-induced hypoxia. This has proven to be the case. Both RAW.12 and RAW.108, were significantly more sensitive to this treatment [38]. Supplementation of the growth medium with 1-AG for several generations prior to hypoxia, restored plasmenylethanolamine and rescued RAW.108 cells, yet did not rescue RAW.12 (in which only the ether bond was restored). Interestingly, we were able to supplement the growth medium with 1-alk-1'-enyl-*sn*-glycerol (1-AEG) and restore plasmenyl-ethanolamine biosynthesis. This compound already contains the vinyl-ether functionality and was apparently able to enter the biosynthetic pathway for plasmenylethanolamine and bypass the deficiency in Δ1'-desaturase activity [38]. Supplementation of the RAW.12 cells with 1-AEG did rescue the cells.

These results very strongly suggest that plasmalogens (the vinyl-ether functionality) are important in protecting these cells against chemically induced anoxia. Whether plasmalogens are serving as an antioxidant or are protecting the cells from other forms of damage is not known yet. Certainly other mechanisms for hypoxia-induced cell injury have been proposed such as the generation of membrane perturbing lipid species such as lysopholipids [4] and free fatty acids [23]. The presence of plasmalogens may affect these processes as well. Still, the known chemistry of the vinyl-ether group and the specific requirement for the vinyl-ether group (and not just the ether bond) in the protective effect suggests the antioxidative role. Obviously more has to be done with this system to determine the exact mechanism of protection by plasmalogen.

Summary

Although a great deal of information exists concerning the biochemistry, distribution, physical nature and chemistry of ether phospholipids, we have yet to positively identify a function for these compounds. The role of plasmalogen in cell function is still a mystery. Why would cells take the effort to synthesize phospholipids which contain a stable ether bond and then spend additional energy to convert that bond to a highly unstable vinyl-ether? Certainly, high levels of ether phospholipids are not required for cell survival in culture (no altered growth phenotypes have been observed in any of the ether lipid-deficient mutants). Perhaps they serve more subtle functions which are required by the intact organism. The ether lipid deficiency disorders described in humans suggest that this may be the case.

The availability of ether lipid-deficient mutants in rapidly growing, immortal cell lines allows us to identify relationships between these lipids and cell function in an isolated, well defined system. The molecular nature of these relationships may then be defined within this system and we may eventually be able to expand our findings to explain the role of ether lipids in functioning of the entire organism and to define the role that their loss plays (if any) in manifestation of symptoms associated with the human peroxisomal/ether lipid disorders.

References

1. Baker, R.C., M. Tucker and K.L. Clay. Ethanol inhibits zymosan-stimulated and enhances nonstimulated platelet-activating factor production in a clonal macrophage cell line. *J. Pharmacol. Exptl. Thera.* **252**, 1028–1033, 1990.
2. Bartlett, P.D., M.S. Mendenhall and A.P. Schaap. Competitive modes of reaction of singlet oxygen. *Ann. N. Y. Acad. Sci.* **171**, 79–88, 1970.
3. Beutler, B., J. Mahoney, N. Le Trang, P. Pekala and A. Cerami. Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. *J. Exp. Med.* **161**, 984–995, 1985.
4. Chien, K.R., J. Abrams, A. Serroni, J.T. Martin and J.I. Farber. Accelerated phospholipid degradation and associated membrane dysfunction in irreversible ischemic liver cell injury. *J. Biol. Chem.* **253**, 4809–4817, 1978.
5. Corey E.J., M.M. Mehrotra and A.U. Khan. Antiarthritic gold compounds effectively quench electronically excited singlet oxygen. *Science* **236**, 68–69, 1987.
6. DaTorre, S.D. and M.H. Creer. Differential turnover of polyunsaturated fatty acids in plasmalogen and diacylglycerophospholipids of isolated cardiac myocytes. *J. Lipid. Res.* **32**, 1159–1172, 1991.
7. Dawson, T.L., G.J. Gores, A.L. Nieminen, B. Herman and J.J. Lemasters. Mitochondria as a source of reactive oxygen species during reductive stress in rat hepatocytes. *Am. J. Physiol.* **264**, C961–C967, 1993.
8. Dembritsky, V.M. Quantitation of plasmalogen, alkylacyl and diacyl glycerophospholipids by micro-thin-layer chromatography. *J. Chromatogr.* **436**, 467–473, 1988.
9. Foote, C.S. **In**: W.A. Pryor (ed.), *Free Radicals in Biology*, **Vol. II**, 85–133, 1976. New York: Academic Press.
10. Gatt, S., J. Bremer and H. Osmundsen. Pyrene dodecanoic acid coenzyme A ester: peroxisomal oxidation and chain shortening. *Biochim. Biophys. Acta* **958**, 130–133, 1988.
11. Gores, G.J., A.L. Nieminen, K.E. Fleishman, T.L. Dawson, B. Herman and J.J. Lemasters. Extracellular acidosis delays the onset of cell death in ATP-depleted hepatocytes. *Am. J. Physiol.* **26**, C347–C354, 1988.
12. Gross, R.W. High plasmalogen and arachidonic acid content of canine myocardial sarcolemma: a fast atom bombardment mass spectroscopic and gas chromatography-mass spectroscopic characterization. *Biochemistry* **23**, 158–165, 1984.
13. Hoefler G., E. Paschke, S. Hoefler, A.B. Moser and H.W. Moser. Photosensitized killing of cultured fibroblasts from patients with peroxisomal disorders due to pyrene fatty acid-mediated ultraviolet damage. *J. Clin. Invest.* **88**, 1873–1879, 1991.
14. Horrocks, L.A. The alk-1-enyl group content of mammalian myelin phosphoglycerides by quantitative two-dimensional thin-layer chromatography. *J. Lipid Res.* **9**, 469–472, 1968.

15. Horrocks, L.A. and M. Sharma. Plasmalogens and O-alkylglycerophospholipids. **In**: J.N. Hawthorne and G.B. Ansell (eds.), *New Comprehensive Biochemistry*, **Vol. 4**, 51–93, 1982. Amsterdam, Elsevier Biomedical Press.
16. Kelley, R.I. The cerebrohepatorenal syndrome of Zellweger, morphological and metabolic aspects, *Amer. J. Med. Gen.* **16**, 503–517, 1983.
17. Lazarow, P.B. and H.W. Moser, 1989. Disorders of peroxsome biogenesis. **In**: C.R. Schriver, A.L. Beaudet, W.S. Sly and D. Valle, (eds.), *The Metabolic Basis f Inherited Disease*, **6th edition**, 1479–1509, 1989. McGraw-Hill.
18. Lohner, K., H. Hermetter and F. Paltauf. Phase behavior of ethanolamine plasmalogen. *Chem. Phys. Lipids* **34**, 163–170, 1984.
19. Morand, O.H., R.A. Zoeller and C.R.H. Raetz. Disappearance of plasmalogens from membranes of animal cells subjected to photosensitized oxidation. *J. Biol. Chem.* **263**, 11597–11606, 1988.
20. Morand, O.H., L.H. Allen, R.A. Zoeller and C.R.H. Raetz. A rapid selection for animal cell mutants with defective peroxisomes. *Biochim. Biophys. Acta* **1034**, 132–141, 1990.
21. Mueller, H.W., J.T. O'Flaherty, D.G. Green, M.P. Samuel and R.L. Wykle. 1-O-alkyl-linked glycerophospholipids of human neutrophils: distribution of arachidonate and other acyl residues in the ether-linked and diacyl species. *J. Lipid Res.* **25**, 383–388, 1984.
22. Owens, K. A two-dimensional thin-layer chromatography procedure for the estimation of plasmalogens. *Biochem. J.* **100**, 354–361, 1966.
23. Sheridan, A.M., J.H. Schwartz, V.M. Kroshian, A.M. Tercyak and W. Lieberthal. Mouse proximal tubular cells are more susceptible than MDCK cells to chemical anoxia. Role of differences in lipid metabolism. *Am. J. Physiol.* **265**, 342–350, 1993.
24. Sigiura, T., M. Makajima, N. Sekiguchi, Y. Nakagawa and K. Waku. Different fatty chain compositions of alkenylacyl, alkylacyl and diacyl phospholipids in rabbit aveolar macrophages: high amounts of arachidonic acid in ether phospholipids. *Lipids* **18**, 125–129, 1983.
25. Smaby, J.M., A. Hermetter, P.C. Schmid, F. Paltauf and H.L. Brockman. Packing of ether and ester phospholipids in monolayers. Evidence for hydrogen-bonded water at the *sn*-1 acyl group of phosphatidylcholines. *Biochemistry* **22**, 5808–5814, 1983.
26. Stevens, V.L. and C.R.H. Raetz. Class F THY-1-negative murine lymphoma cells are deficient in ether lipid biosynthesis. *J. Biol. Chem.* **265**, 15653–15658, 1990.
27. Tence, M., E. Jouvin-Marche, G. Bessou, M. Record and J. Beneviste. Etherphospholipid composition in neutrophils and platelets. *Thromb. Res.* **38**, 207–214, 1985.
28. Tsukamoto, T., S. Miura and Y. Fujiki. Restoration by a 35K membrane protein of peroxisome assembly in a peroxisome-deficient mammalian cell mutant. *Nature* **350**, 77–81, 1991.
29. Turrens, J., Alexandre and A.L. Lehninger. Ubisemiquinone is the electron

donor for superoxide formation by complex III of heart mitochondria. *Arch. Biochem. Biophys.* **237**, 408–411, 1985.
30. Vargaftig, B.B. and J. Beneviste. Platelet activating factor today. *Trends Pharm. Sci.* **4**, 341–343, 1983.
31. Wanders, R.J.A., H. Schumacher, J. Heikoop, R.B.H. Schutgens and J.M. Tager. Human dihydroxyactonephosphate acyltransferase deficiency: a new peroxisomal disorder. *J. Inher. Metab. Dis.* **15**, 389–391, 1992.
32. Zoeller, R.A. and C.R.H. Raetz. Isolation of animal cell mutants deficient in plasmalogen biosynthesis and peroxisome assembly. *Proc. Natl. Acad. Sci. USA* **83**, 5170–5174, 1986.
33. Zoeller, R.A., P.D. Wightman, M.S. Anderson and C.R.H. Raetz. Accumulation of lysophosphatidylinositol in RAW 264.7 macrophage tumor cells stimulated by lipid A precursors. *J. Biol. Chem.* **262**, 17212–17220, 1987.
34. Zoeller, R.A., O.H. Morand and C.R.H. Raetz. A possible role for plasmalogens in protecting animal cells against photosensitized killing. *J. Biol. Chem.* **263**, 11590–11596, 1988.
35. Zoeller, R.A., L.H. Allen, M.L. Santos, P.B. Lazarow, A. Tartakoff, T. Hashimoto and C.R.H. Raetz. Chinese hamster ovary cell mutants defective in peroxisome biogenesis: comparison to Zellweger syndrome. *J. Biol. Chem.* **264**, 21872–21878, 1989.
36. Zoeller, R.A., S. Rangaswamy, H. Herscovitz, W.B. Rizzo, A. Hajra, A.K. Das, H.W. Moser, A. Moser, P.B. Lazarow and M.J. Santos. Mutants in a macrophage-like cell line are defective in plasmalogen biosynthesis, but contain functional peroxisomes. *J. Biol. Chem.* **267**, 8299-8306, 1992.
37. Zoeller, R.A., M.D. Layne and E.J. Modest. Animal cell mutants unable to take up PAF analogues are defective in phagocytosis. Submitted for publication.
38. Zoeller, R.A. and W. Lieberthal. Manuscript in preparation.

Affiliation

Dr. R.A. Zoeller
Boston University School of Medicine
Department of Biophysics
80 East Concord Street
Boston, MA 02118
U.S.A.