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A comparative study of the effect of the antineoplastic ether lipid 1-*O*-octadecyl-2-*O*-methyl-glycero-3-phosphocholine and some homologous compounds on PKCα and PKCε

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Abstract

The effects of the anti-neoplastic ether lipid ET-18-*O*CH_3 and some structural homologues on the activity of protein kinase Cα (PKCα) were studied and compared with the effects the same had on the activity of PKCε. ET-18-*O*CH_3 progressively inhibited the activity of PKCα as the concentration was increased up to 30 mol% of the total lipid, above which the effect was one of activation. The experiments carried out with the homologues showed that the methoxy group bound at the *sn*-2 position of the glycerol of ET-18-*O*CH_3 is essential for both the initial inhibitory effect and the subsequent activation effect. On the other hand, variations in the type of bond linking substitutions in the *sn*-1 position, ether or ester, do not seem to play an important role in determining the activity of the enzyme. The effects were different on PKCε since ET-18-*O*CH_3 had a triphasic effect, activating the enzyme at low concentrations, inhibiting it at slightly higher concentrations and then activating it again at higher concentrations. In this case, when the homologues were used, it was observed that the presence of the methoxy group linked to the *sn*-2 position of glycerol and the type of bond linking substitutions to the *sn*-1 position were important for activating the enzyme, so that only homologues with ester bonds as LPC and PAPC were able to induce the initial activation step in a way similar to ET-18-*O*CH_3. Substitution of the phosphocholine group of ET-18-*O*CH_3 by phosphoserine led to a greater activation of PKCα, an effect that comes from the Ca^{2+}-phospholipid binding site probably because of the specific interaction of this site with the phosphoserine group. The action of ET-18-*O*CH_3 and its homologues, as demonstrated in this paper, may permit the selective inhibition or activation of PKCα and PKCε by using the most suitable range of concentrations.

Keywords: Edelphosine; PKC; Anti-neoplastic lipids; ET-18-*O*CH_3; Apoptosis; Cancer

1. Introduction

Lysophosphatidylcholine (LPC) is produced after the hydrolysis of phosphatidylcholine by phospholipase A_2. Despite being rapidly metabolised, it has been found to have cytotoxic effects [1], a finding that triggered the development of lipidic antitumor ethers, which are synthetic molecules of great metabolic stability unlike LPC. These ether lipids are characterized by an ether bond linking the *sn*-1 position of glycerol with a long hydrocarbon chain [2].

Abbreviations: C16-PAF, 1-*O*-hexadecyl-2-acetyl-3-glycero-3-phosphocholine; ET-18-*O*CH_3, 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine; ET-18-OH, 1-*O*-octadecyl-2-hydroxy-*sn*-glycero-3-phosphocholine; ET-18-H, 1-*O*-octadecyl-*sn*-glycero-3-phosphocholine; ET-18-*O*CH_3serine, 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphoserine; LPC, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine; LUV, large unilamellar vesicles; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine; PS, phosphatidylserine; PAPC, 1-palmitoyl-2-acetyl-*sn*-glycero-3-phosphocholine; ENZYMES (EC): PKC (2.7.1.37)

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All of them are structural homologues of the platelet activation factor (PAF), which is synthetized by the cell, but they lack the easily hydrolyzable ester bond, which is found in the *sn*-2 position of PAF.

The ether lipid, 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine (ET-18-*O*CH_3), belongs to the family of anti-tumor ether lipids homologous with platelet-activating factor (PAF). They differ in that the former lacks the readily hydrolyzable ester moiety present at the *sn*-2 position of PAF. In vitro, ET-18-*O*CH_3 is selectively cytotoxic to cancer cells compared with normal cells [3,4] and has been seen to inhibit the growth of murine and human tumors in experimental systems [5].

The antiproliferative action mechanism of the anti-tumor ether lipids has not been unequivocally established. It is clear that the ether lipids are directly absorbed into cell membranes, which are the primary sites of the action and where they accumulate [5–8]. Several biophysical studies have demonstrated that pure ether lipids form micelles at temperatures above the transition temperature and extend lamellar structures with interdigitating hydrocarbon chains at temperatures below the transition [9–11]. Studies on the interaction of these ether lipids with a membrane model of dipalmitoylphosphatidylcholine have shown that they are able to insert themselves in the bilayer, with their long chains parallel to the membrane bilayer chains [12]. Studies using dielaidoylglycerophosphoethanolamine as membrane model have also demonstrated that ET-18-*O*CH_3 affects the two phase transitions occurring in dielaidoylglycerophosphoethanolamine and increases the *d*-spacing, as detected by X-ray diffraction [13].

Besides acting in the plasma membrane, ether lipids appear to affect tumoral cells in many other ways, affecting the cellular transport systems, promoting cytokine formation, inducing apoptosis, and generally interfering with a wide variety of key enzymes, most of which are membrane-associated and involved with lipid metabolism and/or cell signaling mechanisms [14,15].

Protein kinase C (PKC) is a family of related protein kinases, which includes at least 10 different mammalian isoforms. They play an important role in regulating cell growth as they are involved in several intracellular pathways that lead to transcription. They can be classified into three groups according to their structure and cofactor regulation. The first group includes the classical isoforms (α, βI, βII and γ), which can be distinguished from other groups because their function is regulated by calcium, acidic phospholipids, diacylglycerol and phorbol esters. The second group corresponds to the novel PKCs (δ, ε, η and θ) which are activated by phospholipids, diacylglycerol and phorbol esters, although their function is not regulated by calcium. The third group comprises the atypical PKC isoforms (ξ, τ/λ), which are not regulated by diacylglycerol or calcium [16–18].

Several studies have demonstrated the effect of ET-18-*O*CH_3 and its homologues on PKC. For example, studies performed in leukemic cells suggest that ether-linked lipids compete for activating binding sites on the enzyme, although it was not clear if the competition is for the site corresponding to diacylglycerol or 12-*O*-tetradecanoylphorbol 13-acetate or for that of phosphatidylserine, or even for both of these sites [19,20]. It also inhibits PKC-dependent phosphorylation of endogenous proteins in MCF-7 cells [21] and affects the level of PKC autophosphorylation [22]. On the other hand, some studies have demonstrated that ET-18-*O*CH_3 stimulates PKC activity; for example, the activity of membrane-bound PKC was higher in HL-60 cells treated with ET-18-*O*CH_3 compared with the activity observed in untreated HL-60 cells. Apparently, these differences reside in the way in which the ether and activating phospholipids are added [23]. A recent report comparing the effect on NIH3T3 fibroblasts of soluble ET-18-*O*CH_3 and ET-18-*O*CH_3 incorporated in ELL-12 liposomes [24] demonstrated that, while the soluble form inhibited PKC activity and membrane translocation, the liposome-incorporated form had no effect on either activity or function [25].

More recently, it was shown that a correlation exists between the effect of ET-18-*O*CH_3 on the membrane and the activity of PKCα [26]. These authors observed that when PKCα was activated by lipid vesicles containing DMPC and DMPS, low concentrations of ET-18-*O*CH_3 produced moderate inhibition, while higher concentrations of this lipidic ether induced a clear activation and very high concentrations inhibited. This was interpreted by reference to the effects of the ether on the vesicles, so that at low concentrations the predominant effect was modification of the membrane due to the inverted-cone shape of this molecule. At moderate concentrations, however, the predominant effect was to decrease the size of the vesicles, giving rise to a higher activation of PKC because small vesicles are better activators of PKC. Finally, at very high concentrations, a dilution of PS is the probable reason for inhibition [26]. It was also shown in this paper [26] that ET-18-*O*CH_3 does not interfere with the binding of phorbol ester to PKC.

In this work we used ET-18-*O*CH_3 and some homologues to study their capacity to modulate the activity of PKCα and PKCε, and to correlate any such ability with their pro-apoptotic properties, which have been studied previously [4]. It should be noted that PKCε belongs to the family of novel PKCs, and PKCα, which is a classical PKC, may play an antagonistic role with respect to the regulation of apoptotic processes [27–30].

Our results show that the effects differ since ET-18-*O*CH_3 activates PKCε at low concentrations whereas it inactivates PKCα at the same concentrations. This differential effect of ET-18-*O*CH_3 may have interesting implications for clarifying the action mechanism of these lipidic ethers, while the homologues may be used to selectively inhibit one of these isoenzymes.