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Toxicity of oxidized phosphatidylcholines in cultured human melanoma cells

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ABSTRACT

The oxidized phospholipids (oxPL) 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC) and 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine (POVPC) are generated from 1-palmitoyl-2-arachidonoyl-phosphatidylcholine under conditions of oxidative stress. These oxPL are components of oxidized low density lipoprotein. They are cytotoxic in cells of the arterial wall thus playing an important role in the development and progression of atherosclerosis. The toxic lipid effects include inflammation and under sustained exposure apoptosis. The aim of this study was to find out whether such toxic effects, especially apoptosis, are also elicited by oxPL in melanocytic cells in order to assess their potential for therapeutic intervention.

FACS analysis after staining with fluorescent markers was performed to identify the mode of lipid-induced cell death. Activation of sphingomyelinase which generates apoptotic ceramide was measured using an established fluorescence assay. Ceramide profiles were determined by mass spectrometry.

We found that 50 μM POVPC induce cell death in human melanoma cells isolated from different stages of tumor progression but affect primary human melanocytes to a much lesser extent. In contrast, 50 μM PGPC was only apoptotic in two out of four cell lines used in this study. The toxicity of both compounds was associated with efficient lipid uptake into the tumor cells and activation of acid sphingomyelinase. In several but not all melanoma cell lines used in this study, activation of the sphingomyelin degrading enzyme correlated with an increase in the concentration of the apoptotic mediator ceramide. The individual patterns of the newly formed ceramide species were also cell line-specific.

PGPC and POVPC may be considered potential drug candidates for topical skin cancer treatment. They are toxic in malignant cells. The respective oxidized phospholipids are naturally formed in the body and resistance to these compounds is not likely to occur.

1. Introduction

Like most cancers melanomas constitute a heterogeneous population within one lesion (Quintana et al., 2010; Shackleton et al., 2009). There are hundreds of different genetic alterations that drive cancer cells to grow, migrate, invade and survive (Sauter and Herlyn, 1998; Palmieri et al., 2009). As a consequence of (tumor) heterogeneity blocking just a single signaling pathway

Abbreviations: aSMase, acid sphingomyelinase; BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; BY-PGPE, N-BODIPY-1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphoethanolamine; BY-POVPE, N-BODIPY-1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphoethanolamine; CER, ceramide; FCS, fetal calf serum; NBD, N-7-nitrobenz-2-oxa-1,3-diazole; OxPL, oxidized phospholipid; PBS, phosphate buffered saline; PGPC, 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine; PLPC, 1-palmitoyl-*sn*-glycero-3-phosphocholine; POVPC, 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine; PS, phosphatidylserine; RPMI-1640 medium, Roswell Park Memorial Institute medium; RT, room temperature; SFM, serum-free medium; SM, sphingomyelin; TLC, thin-layer chromatography.

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with an inhibitor is not sufficient to eradicate all cancer cells. Malignant cells within one tumor often differently respond to therapy, some may even remain unaffected. Thus, recent therapeutic strategies are based on the combination of multiple pathway inhibitors, and new concepts have evolved such as individualized therapies and combination therapies (Nikolaou et al., 2012). However, identifying mutations, which can be targeted with molecular inhibitors, for each patient as a personalized approach, is time-consuming and costly. This holds true specifically for early and atypical melanocytic lesions. Because of these reasons, there is a need for a more general approach to target all cancer cells within a tumor independently of their genetic background. Here, we present a novel, generalized strategy which could add to the already existing armamentarium for the treatment of melanocytic disorders, epithelial skin cancer and other cancer types irrespective of their genetic background. It makes use of a subclass of oxidized phospholipids (oxPL) that are formed endogenously in the human body under oxidative stress. The respective compounds are the truncated phosphatidylcholines PGPC and POVPC (see Fig. 1) which contain a long fatty acid and a short carboxylic acid in positions *sn*-1 and -2 of the phospholipid, respectively. We show evidence that 50 μM POVPC preferentially kill melanoma cells in culture as compared to primary human melanocytes. In contrast, 50 μM PGPC were only apoptotic in two out of four cell lines used in this study. In this context, we investigated the toxicity, uptake and target interactions of the cytotoxic compounds in cultured human melanoma cells of different stages. The basis for our study was the observation, that truncated oxPL which are components of oxidized low density lipoprotein (oxLDL) and minimally modified low density lipoprotein (mmLDL) are cytotoxic in cells of the arterial wall thus playing an important role in the development and progression of atherosclerosis (Ross, 1993; Steinberg and Witztum, 1990). The toxic lipid effects include inflammation and under sustained exposure apoptosis.

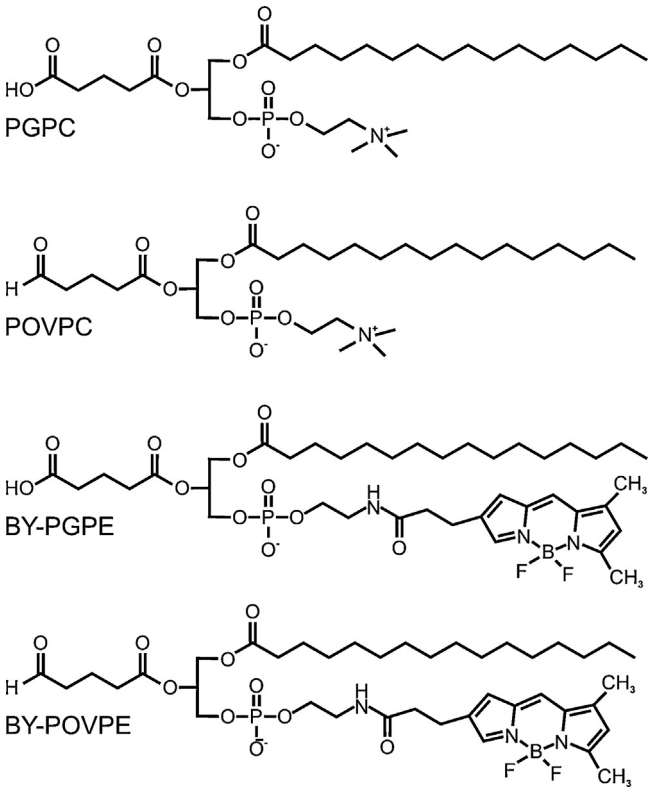

Fig. 1. Chemical structures of oxidized phospholipids.
1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC);
1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine (POVPC);
N-BODIPY-1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphoethanolamine (BY-PGPE);
N-BODIPY-1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphoethanolamine (BY-POVPE).

The aim of this study was to find out whether such toxic effects, especially apoptosis, are also elicited by oxPL in melanocytic cells. We found conditions for pronounced induction of cell death in human melanoma cell lines leaving primary human melanocytes nearly unaffected. The toxicity of PGPC and POVPC was associated with efficient uptake of these lipids into the cells, activation of aSMase and the formation of the apoptotic second messenger ceramide. This suggests that oxPL might possess high potential for cancer treatment because their toxicity can be preferentially directed toward malignant cells.

2. Materials & methods

2.1. Cell culture and incubation with oxPL

Human primary melanoma cell lines (SBcl2, WM35) and metastatic melanoma cell lines (WM9, WM164) were cultured in RPMI-1640 medium with 2% FCS, 200 units/ml penicillin/streptomycin and 4 mM L-glutamine. Human melanocytes FOM, derived from human foreskin, were cultured in Melanocyte Growth Medium (MGM). The melanocytic cell lines were kindly provided by Dr. Meenhard Herlyn (The Wistar Institute, Philadelphia, PA, USA). All cells were grown at 37 °C in humidified CO_2 (5%) atmosphere. Phospholipid dispersions in aqueous media (see below) were prepared using the ethanol injection method (Batzri and Korn, 1973). The final concentration of EtOH was always kept below 1% (v/v). Incubation medium for all experiments was RPMI-1640 culture medium containing 0.1% FCS without phenol red unless otherwise indicated. Control experiments were carried out with incubation medium containing 0.1% FCS and the same concentration of EtOH but without lipids.

2.2. Fluorescence microscopy

Cells were grown to 70% confluence on chamber slides (Nunc, Nalgene, Rochester USA). Cells were incubated with dispersions of 5 μM fluorescent oxPL in incubation medium for 5 and 30 min (fluorescent oxPL are availaible from Avanti Polar Lipids, Alabaster, AL, USA). After incubation, cells were washed with medium and observed with an Axiovert 35 inverted fluorescence microscope equipped with a mercury-arc lamp and a CCD camera, driven by AxioVision software package (Carl Zeiss, Germany). BODIPY fluorescence (Ex 505 nm, Em 510 nm) was detected using the following filter set: Excitation filter BP 450–490 nm, Beam splitter 510 nm and barrier filter LP 520 nm. Unlabeled cells were used as a reference to examine autofluorescence.

2.3. FACS analysis

For the determination of apoptotic and necrotic cell populations, the Vybrant® apoptosis assay kit#2 was used as previously described (Stemmer et al., 2012a). Each experiment was carried out three times and each sample was done in parallel.

2.4. Morphological studies

Cells were grown to 60–70% confluence in chamber slides (Nunc, Nalgene, Rochester, USA), incubated with oxPL or 1% EtOH (v/v) in media without phenol red under low serum conditions and observed under the microscope as previously described (Stemmer et al., 2012a).