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Caged ceramide 1-phosphate (C1P) analogs: Novel tools for studying C1P biology

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ABSTRACT

Ceramide 1-phosphate (C1P) is a bioactive sphingolipid metabolite that is produced in cells by the action of ceramide kinase (CerK) acting upon ceramide, and is also found in the circulation. C1P was first demonstrated to be mitogenic and antiapoptotic in different cell types, and was later shown to induce cell migration. Understanding the precise mechanisms by which C1P exerts its biological effects has been possible using specific photosensitive caged C1P analogues synthesized by Robert Bittman's group. These compounds are cell permeable, bypass cell plasma membrane receptors, and can be released into the cytosol upon light irradiation, thereby allowing precise determination of the intracellular mechanisms of actions of C1P. Two derivatives of *N*-palmitoyl-ceramide 1-phosphate have been used in most studies. In one C1P derivative the cage was 7-(*N*,*N*-diethylamino)coumarin (DECM-C1P) while in the other it was a 4-bromo-5-hydroxy-2-nitrobenzhydryl moiety (BHNB-C1P). The uncaging process released C1P in the cytosol, and this was accompanied by stimulation of cell proliferation, inhibition of apoptosis, and production of low levels of reactive oxygen species. However, intracellular accumulation of C1P did not affect chemotaxis. The caged C1P analogues allowed distinction between the extracellular events evoked by C1P, as for example through interaction with a putative cell-surface receptor, from its intracellular effects.

1. Introduction

The discovery in the late 1980s and early 1990s that ceramides served as second messengers for regulation of cell differentiation and homeostasis (Bielawska et al., 1992; Kim et al., 1991; Kolesnick, 1992, 1991; Mathias et al., 1993; Obeid et al., 1993; Okazaki et al., 1989, 1990; Wang et al., 1991; Zhang et al., 1997) marked the beginning of a new era in cell biology research. Initial studies showed that ceramides were potent inhibitors of cell growth and inducers of apoptosis, and these findings have been extensively confirmed by many groups, including ours, in a variety of cell types (reviewed in (Gomez-Munoz, 1998; Hannun and Bell, 1993; Hannun and Linardic, 1993; Hannun et al., 1993; Kolesnick, 1992, 1994; Kolesnick and Golde, 1994; Merrill, 1992; Merrill et al., 1993; Merrill and Schroeder, 1993). Subsequent studies on ceramide metabolism led to the discovery of ceramide kinase, the enzyme responsible for conversion of ceramide to ceramide 1-phosphate (C1P) (Bajjalieh et al., 1989). This novel sphingolipid metabolite was first observed in human leukemia HL-60 cells (Dressler and Kolesnick, 1990), but no biological activity was associated to it, at the time. The bioactivity of C1P was first demonstrated using rat-2 fibroblasts as experimental model for studying cell growth and death. Unexpectedly, and contrary to ceramides, C1P resulted to be a potent stimulator of cell growth when added exogenously to cultured cells (Gomez-Munoz et al., 1995). The latter studies were carried out using short-chain C1P analogues, mainly *N*-acetyl-ceramide 1-phosphate (C2-C1P) and *N*-octanoyl-ceramide 1-phosphate (C8-C1P) that were synthesized by Bittman and co-workers (Gomez-Munoz et al., 1995). The cell permeability of these synthetic short chain analogs was superior to that of natural long-chain C1P, which resulted to be critical for demonstrating that C1P had mitogenic properties in fibroblasts. Subsequent studies showed that other cell types, including primary

Abbreviations: Akt (PKB), protein kinase; B BHNB, 4-bromo-5-hydroxy-2-nitrobenzhydryl; BMDM, bone marrow-derived macrophages; CerK, ceramide kinase; C1P, ceramide 1-phosphate; cPLA2, calcium-dependent cytosolic phospholipase 2; DECM, 7-(*N*,*N*-diethylamino)coumarin; ERK, extracellularly regulated kinases; GLUT, glucose transporter; iNOS, inducible nitric oxide synthase; PKC, protein kinase C; PI3K, phosphatidylinositol 3-kinase; PTX, pertussis toxin; ROS, reacfive oxygen species; SMase, sphingomyelinase; SPT, serine palmitoyl transferase.

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bone marrow-derived macrophages (BMDM), were responsive to challenges with natural long-chain C1P. In particular, *N*-palmitoyl-ceramide 1-phosphate (C16-C1P), or a natural mixture of bovine brain-derived C1P with *N*-linked fatty acid chains composed mainly of stearic and nervonic acids, were as potent as C8-C1P at stimulating macrophage growth (Gangoiti et al., 2011; Gangoiti et al., 2010a; Gangoiti et al., 2008b). In addition to demonstrating that C1P is mitogenic, we showed that both synthetic short chain C1Ps and natural long-chain C1P were able to inhibit apoptosis (Gangoiti et al., 2008a; Gomez-Munoz et al., 2005, 2004; Granado et al., 2009a), and to stimulate cell migration in different cell types (Arana et al., 2013; Granado et al., 2009b; Ouro et al., 2014). In this connection, it should be emphasized that C1P has dual actions on cells, being able to act intra- or extra-cellularly to exert its biological effects. Induction of intracellular accumulation of C1P may not be difficult in cells that can be easily transfected with appropriate ceramide kinase (CerK) plasmids; however, many cell types, including primary BMDM, are fairly resistant to transfection or cannot be efficiently transfected (Comalada et al., 2004). In these particular cases alternative strategies to increase the levels of C1P intracellularly are needed. One of these strategies was recently developed by Bittman and co-workers, who designed cell permeable caged C1P analogs to specifically increase C1P concentrations inside cells. These compounds facilitate the studies on the intracellular actions of C1P, and can help to identify the intracellular targets of C1P in a precise manner.

The present review highlights the importance of using photosensitive caged C1P analogues to distinguish between the intra- and extracellular actions of C1P.

2. C1P biology

It has been clearly established that C1P can act both intracellularly and through interaction with a putative plasma membrane receptor (reviewed in (Gomez-Munoz et al., 2013)). C1P has been shown to regulate numerous cell functions including cell proliferation, apoptosis, cell migration, arachidonic acid release, mast cell degranulation, calcium mobilization, or glucose uptake and glycolisis (Arana et al., 2013; Chalfant and Spiegel, 2005; Gangoiti et al., 2011, 2008b; Gomez-Munoz, 2006; Gomez-Munoz et al., 2013; Granado et al., 2009b; Hinkovska-Galcheva et al., 2005; Lamour and Chalfant, 2005; Mitsutake et al., 2004; Ouro et al., 2013; Ratajczak et al., 2013; Wijesinghe et al., 2007). Some of these effects involved translocation of key regulatory proteins or enzymes from the cytosol to specific membrane compartments, or direct interaction of C1P with specific proteins. For instance, the stimulation of cell proliferation by C1P required translocation of protein kinase C-alpha (PKC-α) from the cytosol to the microsomal fraction in primary macrophages (Gangoiti et al., 2010b). Also, translocation of the glucose transporter GLUT3 was required for the stimulation of glucose uptake by C1P in RAW264.7 macrophages, suggesting that C1P is a regulator of carbohydrate metabolism in these cells (Ouro et al., 2013). The mechanism whereby C1P promoted glucose uptake in macrophages involved enhancement of the affinity of the GLUT3 transporter for glucose as well as stimulation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, also known as Akt), pathway (Ouro et al., 2013). Furthermore, C1P stimulated the production of reactive oxygen species (ROS) by promoting the translocation of the NADPH oxidase subunit p47phox from the cytosol to the plasma membrane of cells, an action that facilitated phosphorylation of p47phox and the subsequent activation of NADPH oxidase leading to ROS production in these cells (Arana et al., 2012). Although generation of ROS has been mainly associated to induction of apoptosis in a variety of cells types (Finkel and Holbrook, 2000; Manea, 2010; Novo and Parola, 2008; Sharma et al., 2012; Syed et al., 2011), the increased levels of ROS by C1P in macrophages was relative small (1.6–1.8 fold over the control value) and was not associated to induction of cell death. On the contrary, these low concentrations of ROS

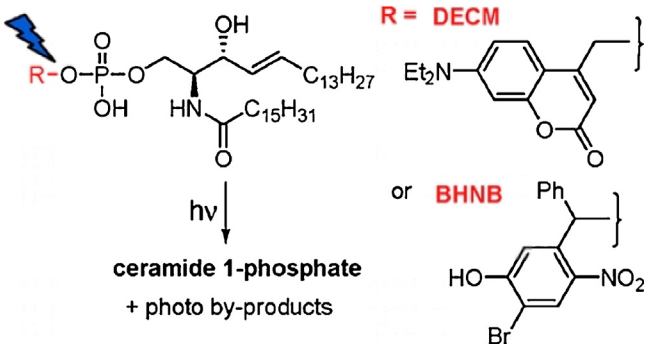

Fig. 1. Sphingolipid phosphate analogues bearing 7-(diethylamino)-coumarin (DECM) and 4-bromo-5-hydroxy-2-nitrobenzhydryl (BHNB) groups in a photolabile ester bond (taken from Lankalapalli et al., 2009; with permission).

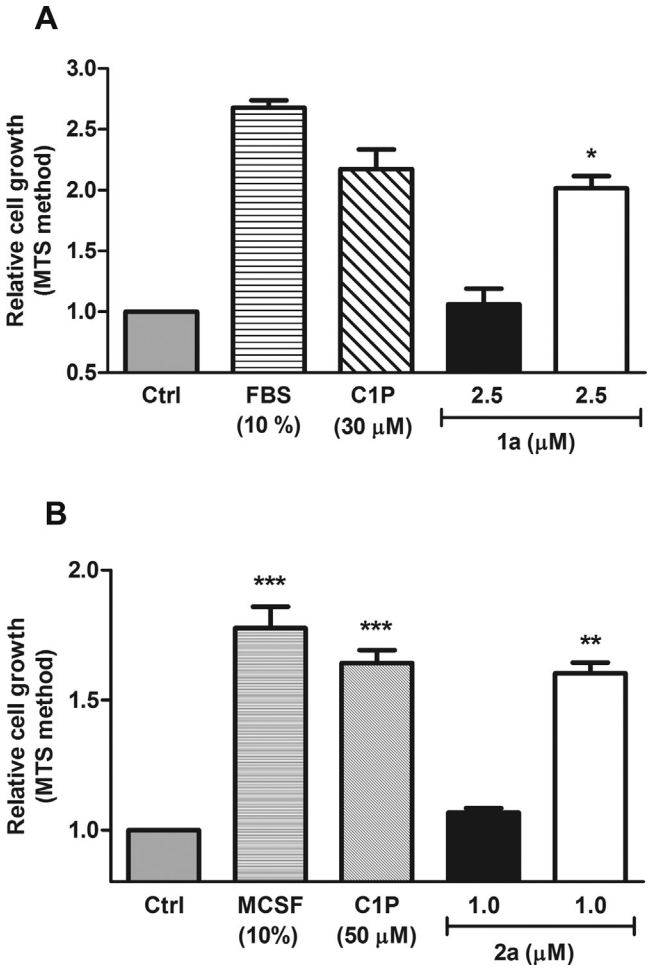

Fig. 2. Delivery of DECM-C1P (1a) and BHNB-C1P (2a) into RAW 264.7 macrophages (panel A) and primary bone marrow derived macrophages (panel B) stimulates cell growth. *Open bars*: after the cells were incubated with compound 1a or 2a in the dark for 30 min, the cells were exposed to 400–500 nm light for 60 min in a dark reader non-UV transilluminator (DR45M from Clare Chemical Research, Denver, CO, USA) equipped with a 9 W lamp. *Filled bars*: the cells were incubated in the dark with the compounds. (A) The cells were incubated for 48 h in the absence of FBS or (B) for 24 h with 1.5% macrophage colony stimulating factor (MCSF) prior to the addition of the compounds. Mean ± S.D. of 3 independent experiments. (Taken from Lankalapalli et al., 2009; with permission).